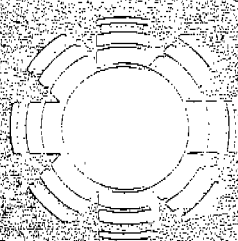
SERI/SP-231-3331
DE88001172

July 1988

FY 1987 Anaerobic Digestion

Annual Report



SERI

Solar Energy Research Institute
A Division of Midwest Research Institute
1617 Cole Boulevard
Golden, Colorado 80401-3393

Operated for the
U.S. Department of Energy
Under Contract No. DE-AC02-83CH10093

SERI/SP-231-3331
UC Category: 246, 249
DE88001172

FY 1987 Anaerobic Digestion

Annual Report

July 1988

Prepared under Task No. BF731010

Solar Energy Research Institute
A Division of Midwest Research Institute

1617 Cole Boulevard
Golden, Colorado 80401-3393

Prepared for the
U.S. Department of Energy
Contract No. DE-AC02-83CH10093


SERI

Printed in the United States of America
Available from:
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161

Price: Microfiche A01
Printed Copy A11

Codes are used for pricing all publications. The code is determined by the number of pages in the publication. Information pertaining to the pricing codes can be found in the current issue of the following publications, which are generally available in most libraries: *Energy Research Abstracts, (ERA); Government Reports Announcements* and Index (*GRA* and I); *Scientific and Technical Abstract Reports (STAR);* and publication, NTIS-PR-360 available from NTIS at the above address.

TABLE OF CONTENTS

TABLE OF CONTENTS (Concluded)

Program Overview

OVERVIEW OF THE ANAEROBIC DIGESTION RESEARCH PROGRAM

Barbara J. Goodman
Solar Energy Research Institute
Golden, Colorado 80401

ABSTRACT

Anaerobic digestion research is being undertaken by the Department of Energy (DOE) under the field management of the Solar Energy Research Institute (SERI) and is sponsored as part of the Energy from Municipal Waste Research Program to produce methane from biomass and waste. Primary focus is on municipal solid waste (MSW) as a feedstock; however, some efforts are ongoing using other biomass feedstocks. MSW represents a vast resource for energy production; however, its variability presents unique problems in using it as a feedstock.

To make methane production from waste and biomass economical, researchers are addressing three major technological improvements: (1) increasing solids loading, (2) decreasing solids residence time, and (3) improving conversion efficiency. Improvements have been made in all three areas through biological and engineering research activities and have resulted in a decrease in methane production cost from approximately $8.00/million Btu in 1980 to $5.00/million Btu today.

Future research will continue to increase our understanding of the organisms involved in the anaerobic digestion process and their interactions. Both biological and engineering research will continue in order to decrease the cost of methane to less than $3.50/million Btu by the year 2000.

OVERVIEW OF THE ANAEROBIC DIGESTION RESEARCH PROGRAM

INTRODUCTION

As part of the Energy from Municipal Waste Program, research is being conducted in anaerobic digestion to produce methane from biomass and waste. The primary feedstock on which research efforts currently focus is municipal solid waste (MSW); however, other biomass feedstocks are also considered. MSW is generated by the domestic, institutional, and commercial sectors of the country. Approximately 250 million tons of MSW are discarded each year in the United States, representing about 1.5 - 2.0 Quads of energy. Billions of tons of MSW are currently buried in landfills across the country and are naturally producing methane in an uncontrolled process. In addition, municipalities use approximately 0.2 Quad of energy each year in wastewater treatment. The overall goal of this research is to maximize utilization of this vast resource for energy production.

MSW is very heterogeneous and varies in composition from day to day, season to season, and location to location. Figure 1 gives average numbers for the components typically found in MSW. The combustibles, which include paper, plastic, food, and yard wastes, comprise about 80% of MSW. The remaining 20% is noncombustible and can generally be separated by conventional techniques. The "other" fraction can pose difficulty in utilizing MSW for energy production because its composition is unknown and can include paint, grease, or other substances toxic to microorganisms.

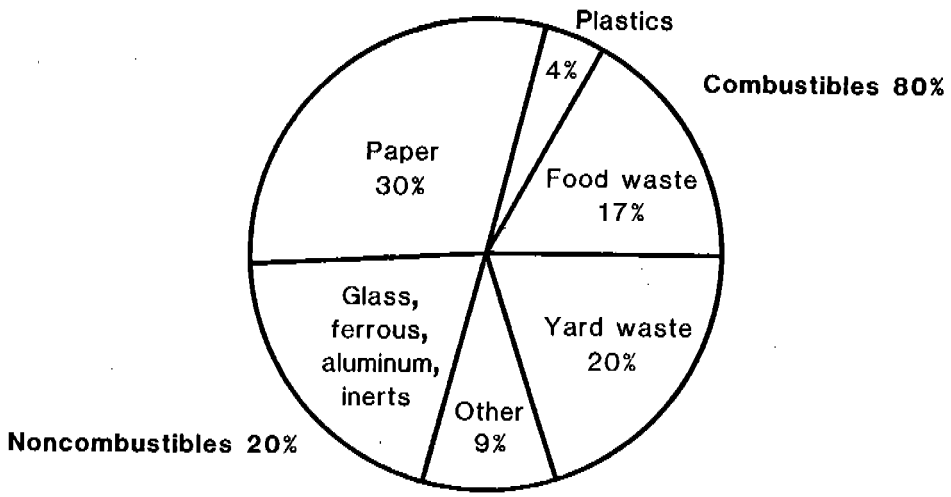


Figure 1. Composition of MSW

There are several options for energy recovery from MSW as depicted in Figure 2. In the past, we typically have deposited trash in landfills without consideration of the consequences. Recently, however, we are tapping these landfills to recover the methane being naturally produced. Another option has been to burn the waste to produce steam and/or electricity. We are currently looking at ways to mechanically separate metals and glass from the waste and process the rest to produce refuse derived fuel (RDF) in either a fluff, powder, or densified form. The RDF can then be used in either a biochemical or thermochemical conversion process to produce energy products.

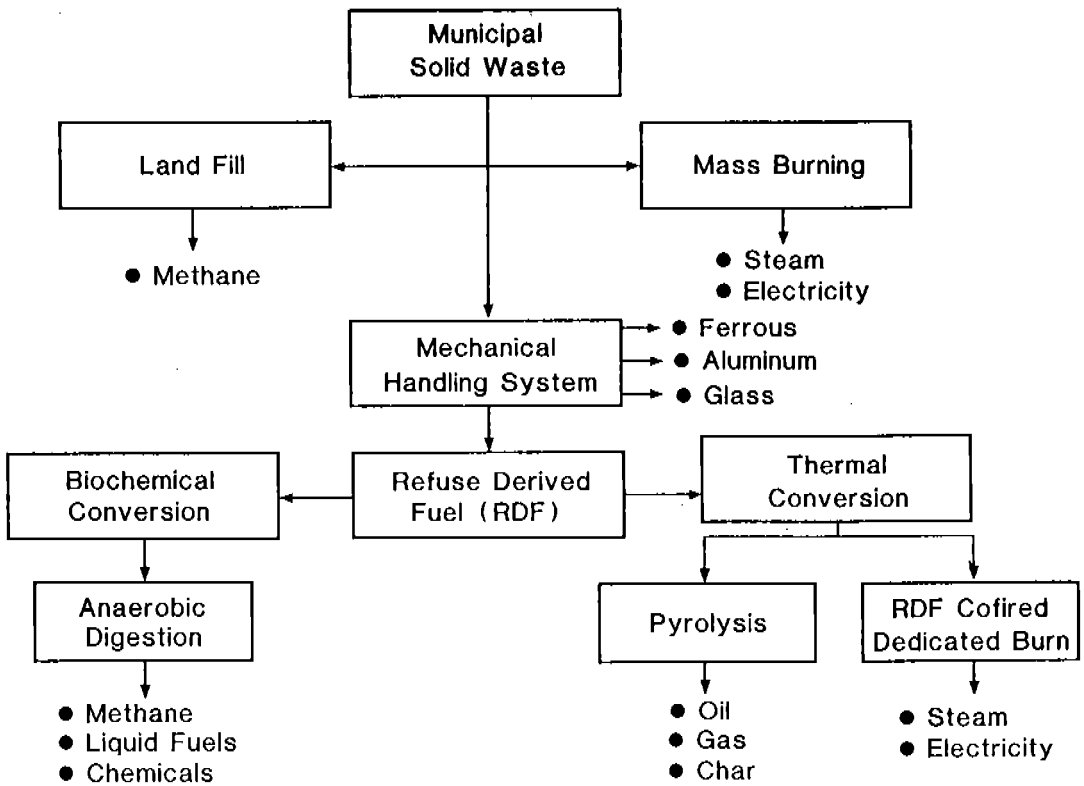


Figure 2. Energy Recovery Options

BACKGROUND

Anaerobic digestion is the most frequently considered biochemical conversion process for MSW. A naturally occurring biological process in landfills, anaerobic digestion is extremely complex and involves numerous groups of organisms acting synergistically to convert complex organic molecules to methane and carbon dioxide. Although methane formation by biochemical means was observed as early as the 19th century, the organisms involved in the process have yet to be adequately classified and characterized. Anaerobes are extremely difficult to study since they require special handling equipment as well as a completely anaerobic environment. Therefore, isolation and characterization of these organisms is a slow process.

The coordinated activity of the microorganisms involved in anaerobic digestion ensures stability during the process. Environmental parameters that influence the activity of one group of organisms can indirectly affect the entire bacterial population, causing digester

failure. The methanogens and acetogens are reported to exhibit the slowest growth rates and can result in "wash out" under short retention times.

In order to utilize MSW in an anaerobic digestion system, processing of the waste, including size reduction and separation of metals, glass, and other inorganic material is necessary. The resulting material is called fluff RDF. Sewage sludge and nutrients are added to the RDF and fed to the anaerobic digester. The residue from the process is dewatered and the liquids are recycled while the solids are combusted to provide steam for heating the digester. The gas is scrubbed to remove carbon dioxide producing a relatively pure methane product. Figure 3 depicts a sample process flow diagram for anaerobic digestion of MSW.

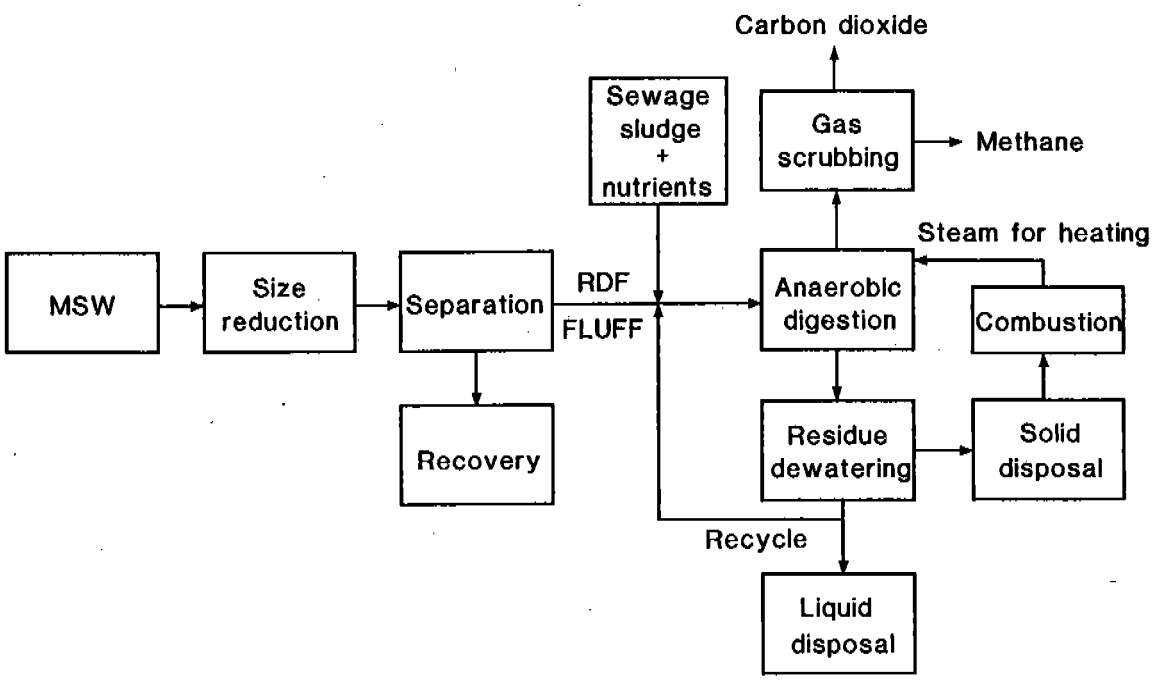


Figure 3. Schematic for Anaerobic Digestion of MSW

PROGRAM GOALS

The overall goal of anaerobic digestion research is to produce methane from waste or biomass for less than $3.50/million Btu by the year 2000. In order to economically produce methane three major technological improvements must be made: (1) increase solids loading, (2) decrease solids residence time, and (3) improve conversion efficiency. Specific research goals for each of these improvements are shown in Table 1.

Table 1. Research Goals

Key Parameters	1980	1987	Goal
Solids concentration in digester (%)	10	10	20-30
Solids residence time in digester (d)	30	25	5
Conversion efficiency (%)	50	55	80
Methane cost ($/million Btu)	8.00	5.00	3.50

CURRENT RESEARCH PROGRAM

For purposes of this paper, research activities in anaerobic digestion will be presented in four major categories as follows:

- Enhanced Bioconversion Research
- Process Engineering Experiments
- Improved Reactor Concepts
- Wastewater Treatment and Landfill Gas Recovery

Detailed descriptions of ongoing research activities in each of these categories are included in this report. Table 2 shows the research efforts currently under way in each of these areas, including their location and the principal investigator.

Enhanced bioconversion research focuses on projects that increase the rates or yields of methane production through understanding the basic biochemistry and physiology of the organisms within a reactor. Research in this area is being conducted at SERI and UCLA. At the University of Kansas and the New York State Department of Health, researchers are investigating means of tracking organisms and improving reactor stability. The exact nature of the interrelationships among sets of microbes is unknown. Research is being conducted to identify and characterize these organisms in order to optimize the process. Factors are being identified that destabilize normally functional digesters. After identifying the cause of failure, it will be possible to design control systems that will allow digesters to operate more efficiently. Genetic improvements are being investigated at Purdue University to increase the conversion efficiency of the anaerobic digestion process.

Process engineering experiments are being performed at the Experimental Test Unit (ETU) at Walt Disney World in Orlando, Florida, and supporting laboratory-scale tests are being conducted at the University of Florida. Research at the ETU has been ongoing for several years; however, DOE is cofunding work with the Gas Research Institute (GRI) and Southern California Edison (SCE) with emphasis on biogas production. As part of this effort, systems modeling and economic analyses are being performed by Reynolds, Smith, and Hill.

Table 2. Current Research Efforts

Project	Location	Principal Investigator
Enhanced Bioconversion Research		
Extracellular Hydrolytic Enzymes	SERI	Mike Himmel
Photoenhanced Anaerobic Digestion	SERI	Paul Weaver
Effects of Molecular Hydrogen on Acetate Degradation by Methanogens	UCLA	Bob Mah
Enzymatic and Initial Genetic Studies on Methanogens	Purdue University	Nancy Ho
Effects of Trace Metals on Methanogenesis and Control of Sulfate Reducing Bacteria	U. of Kansas	Ross McKinney
Biological Conversion of Gasified MSW	SERI	Paul Weaver
Monoclonal Antibodies for Hydrolytic Microbes	New York State Dept. of Health	Everly Conway de Macario
Process Engineering Experiments		
Biogasification of Waste	Walt Disney Imagineering	Ben Schwegler
ETU	IGT	Rich Biljetina
Laboratory Support and Future Plans for ETU	U. of Florida	Dave Chynoweth
Disney Systems and Economic Model	Reynolds, Smith, and Hill	Bob LeGrande
Improved Reactor Concepts		
High Solids Digestion of MSW to Methane	U. of Arkansas	James Gaddy
Pretreated High-Solid MSW for Anaerobic Digestion to Methane	Purdue University	George Tsao
High Solids Fermentation	SERI	Chris Rivard

Table 2. Current Research Efforts (Concluded)

Bioconversion of MSW and Recovery of Short-Chain Acids	ANL	Tony Antonopoulos
Redox Fermentations	SERI	Chris Rivard
Wastewater Treatment & Landfill Gas Recovery		
Variations in Gas Pressures and Concentrations in Landfill Cover Materials	ANL	Jean Bogner
Pilot Study on Microbial Populations in Landfills	ANL	Jean Bogner
Effluent Dewatering Research for MSW and Wastewater Treatment Sludges	ANL	Vic Pearson
Research into Factors Determining Anaerobic Digester Performance	Drexel University	Richard Speece

Improved reactor concepts focus on designs for processing higher concentrations of solids in anaerobic digestion systems. Several systems are being investigated by researchers at the University of Arkansas, Purdue University, and SERI. Typical solids concentration in anaerobic digesters is 6%-10%. Increasing the solids concentration to 20% or 30% would proportionally reduce the digester size required and the resulting gas cost. Both mechanical and mass transfer problems need to be overcome in order to operate high solids reactors while maintaining constant gas production rates. New mixing techniques are being explored to keep the substrate from separating and to keep the microbial population in close contact with the substrate nutrients.

The technologies, processes, and environmental issues associated with methane production from MSW are also applicable to landfill gas recovery processes and wastewater treatment. Research is being pursued in the area of landfill gas recovery at Argonne National Laboratory to understand the effect of atmospheric conditions on the flow of gas and to evaluate the microbial populations that produce landfill gas. Wastewater treatment research is ongoing at Argonne National Laboratory and Drexel University to evaluate the effects of sludge dewatering and to improve the performance of anaerobic digesters for wastewater treatment.

FUTURE RESEARCH DIRECTION

DOE-sponsored research has increased the understanding of the complex interactions of organisms involved in anaerobic digestion. Conversion efficiency has increased while solids residence time has decreased. These factors contributed to reducing the cost of methane produced from anaerobic digestion from approximately $8.00/million Btu in

1980 to $5.00/million Btu using technology available today. Continued research should result in further reductions in methane cost to approximately $3.50/million Btu.

During the next five years, research will continue to identify the organisms present in anaerobic digesters and clarify their complex interactions. Biological research will focus on selecting specific organisms for genetic breeding and manipulation to allow operation of anaerobic digesters at optimal conditions. Feedback and control mechanisms will be developed to maintain stable operation of digesters. Engineering efforts will focus on development of large-scale and new reactor designs that can process high concentrations of solids in order to further decrease the cost of producing methane.

Enhanced Bioconversion Research

DETECTION OF EXTRA-CELLULAR HYDROLYTIC ENZYMES IN ANAEROBIC DIGESTION OF MSW

M.E. Himmel, W.S. Adney, C.J. Rivard and K. Grohmann
Fermentation Section, Biotechnology Research Branch,
Solar Fuels Division, SERI
1617 Cole Blvd, Golden CO 80401

ABSTRACT

In order to develop the maximum conversion rates possible in the production of methane from MSW feedstocks, the slow rate of polymer hydrolysis must be enhanced. This requires an understanding of the important controlling factors governing enzyme production and action in the anaerobic digestion system.

This study reports the adaptation of 16 assays for hydrolytic enzymes to the clarified supernatant obtained from digester sludge. From this battery of assays tested, only two activities, alpha-amylase and a general protease, were detectable in supernatant samples from healthy lab-scale digesters fed MSW meal. The particulates present in anaerobic digesters are now thought to harbor the polymer-binding hydrolytic enzymes important to efficient digester operation. This material was also examined for associated enzyme activity by developing a series of detergent extractions designed to release fully active enzymes from the sludge. Recent studies have shown that using this protocol, cellulase, beta-glucosidase, protease and alpha-amylase activities can be recovered from sludge samples taken from small scale digesters. Also, enzyme activities both from digester supernatant and sludge samples were shown to be oxygen insensitive, an issue important to conducting routine assays.

Now that the analytical methods are available, assays for hydrolytic enzymes can be used to monitor chemical and metabolic fluxes, as they relate to enzyme activity, in the anaerobic digestion of MSW.

DETECTION OF EXTRA-CELLULAR HYDROLYTIC ENZYMES IN ANAEROBIC DIGESTION OF MSW

INTRODUCTION

The extracellular hydrolytic enzymes present in anaerobic digesters treating municipal solid wastes have not been rigorously studied [Khan and van den Berg, 1981]. Indeed, enzyme analysis and detection in the contiguous field of soil ecology, of interest due a similar requirement for optimization of hydrolytic enzyme activity in the presence of a complex chemical and microbiological environment, is also in a state of infancy [Tate and Klein, 1985]. The breakdown of polymers (such as cellulose) in MSW feedstocks has been identified as one of the limiting steps in the overall rate of conversion to methane in the anaerobic digestion process. Research resulting in increased understanding of the enzymes naturally produced in these systems will lead to methods of increasing the hydrolytic power of the anaerobic digestion process by, for example, inoculation with specialized enzyme producers when necessary. This point is important, as a changes in feedstock can result in the introduction of an inhibiting agent or toxin which alters the effectiveness of the current consortium or the levels of enzymes they produce or both.

These polymer degrading enzymes are present in the digester in two general fractions, the first fraction, representing the free-solution (effluent) enzymes, is more readily analyzed by standard methods and was characterized in FY1986. The second enzyme fraction, the solids-bound fraction, is quite inaccessible to current methods of enzyme activity analysis. To add to the importance of this problem, it is now recognized that the most potent or active polymer degrading enzymes in the system are tightly attached to the solids and are not in solution, as these enzymes posses the high binding affinity necessary for specific and rapid breakdown of polymeric substrates.

This report summarizes the work conducted at SERI in FY1987 to establish methods for analyzing the key hydrolytic enzyme activities found both in the effluent and in the solids-bound fraction taken from the SERI laboratory scale anaerobic digesters operated at steady-state on a representative MSW feed.

MATERIALS AND METHODS

Substrates, Enzymes and Detergents

The p-nitrophenyl-compounds, dye-linked polymers, and oat spelts xylan used as substrates for enzyme assays were obtained from Sigma Chemical Co. Whatman No. 1 filter paper was used as substrate for the filter paper assay and carboxymethylcellulose (7LF) from Hercules, Inc. was used for the CMCase assays. The casein-clearing assay kit for general protease activity was obtained from Bio-Rad. The hydrolytic enzymes, porcine pancreas alpha-amylase [EC 3.2.1.1.] (2830 units/mg protein from starch assay at pH 6.9 at 20°C [Bernfeld, 1955]) and bovine pancreas alpha-chymotrypsin [EC 3.4.21.1] (46 units/mg solid using the BTEE ester at pH 7.8 at 25°C), used for standard curves were also obtained from Sigma. Cultures of the cellulase producing bacterium Acidothermus cellulolyticus were prepared in-house as described by Mohagheghi et al. (1986).

The non-ionic and ionic detergents were obtained from Sigma. Zwittergent detergents were obtained from Calbiochem. All buffer components and salts used where of reagent grade and obtained from either Sigma or Fisher Scientific. Remazolbrilliant Blue R dye was from Sigma.

Instrumental

Enzyme assays performed anaerobically were conducted in a 4'x6' Coy Inc. isolation hood maintained at a gas mixture of 90% N_2 and 10% H_2. This hood was equipped with automatic oxygen detection. Colorimetric products from enzyme assays were detected and recorded using a B&L 601 spectrophotometer equipped with sipper and data printer.

A variety of filter cassettes where examined for adsorption properties to determine suitability for removing unreacted dye-substrate following exposure to enzymes. These 0.22 micron filters where obtained from Millipore, Rainin Instruments, and Gelman Scientific.

Enzyme Assays

Assays for endo-beta-1,4 glucanase (carboxymethyl-cellulose degrading, CMCase) and saccharifying cellulase (filter paper units, FPU) activities followed partially the methods of Sternberg, Vijaykumar, and Reese (1977) and Mandels, Andreotti, and Roche (1976), as modified in the 1987 IUPAC report (Ghose et al 1987). Under the recommended conditions of the CMCase and FPU assays, enzyme dilutions must be adjusted so that 0.5 mg and 2.0 mg glucose is released from 10 mg carboxymethylcellulose and 50 mg strips of Whatman No. 1 filter paper after 30 minute and one hour incubations at 50°C, respectively. However, in the present study incubation times ranged from 30 minutes to several hours and the temperature of incubation was that of the digester, 37°C. Since in this study enzyme activities are found directly from the amount of glucose released and not from the required enzyme dilutions as in the IUPAC recommendations, the enzyme activities from digester samples are referred to as "apparent" cellulase activities. Cellulase activities were also estimated by the method of Fernley (1963) using cellulose azure as substrate.

General xylanase activity was estimated by the xylose released from oat spelts xylan in 30 minutes at 37°C following the method of Reilly (1981). Xylose released during this incubation was determined by the DNS method of Miller (1959).

Beta-glucosidase was determined according to the method of Wood (1971) as aryl-beta-glucosidase by the hydrolysis of p-nitrophenyl-beta-glucopyranoside, however, for these studies the incubation buffer was 100 mM Tris pH 7.5. The concentration of p-nitrophenol was determined from the extinction at 410 nm under alkaline conditions induced by the addition of 2 M Na_2CO_3. One unit of activity is defined as that amount of enzyme that catalyzes the cleavage of 1.0 micromol substrate per minute at 37°C. Other aryl-glycosidase activities were determined in a similar way using p-nitrophenyl-substrates. The substrates p-nitrophenyl-palmitate and pNP-stearate were used to detect general lipase activity by the method of Yeoh et al (1986).

Azure-bound polymeric substrates were used to monitor starch, amylose, hide powder and keratin degrading enzymes. The method, modified from that of Rinderknecht et al. (1967) and Bergmeyer (1974), requires a one hour incubation of enzyme sample with 10 mg of insoluble substrate in two mL 100 mM Tris buffer pH 7.5 at 37°C. The dye release is stopped by filtration using 0.22 micron filter cassettes and the free dye is determined at 620 nm. Azocasein hydrolysis was also used to monitor protease activity, here the free dye is determined at 440 nm after alkaline induced precipitation has been used to remove the uncleaved substrate (Bergmeyer 1974).

The commercial cellulase enzyme preparation used to standardize the cellulose-azure substrate and to test detergent effects was the Genencor 150L lot no. 3-86189 (code 6-5919) cellulase, found earlier (Himmel et al. 1986) by the IUPAC methods to have 109 FPU, 2842 CMCU, 288 beta-glucosidase units and 4.3 beta-xylosidase units of activity per mL.

Evaluation of Filtration Cassettes

Comparison of disposable commercial filtration cassettes was done by filtering a 300 ug solution of Remazolbrilliant Blue through cassettes obtained from various manufacturers and comparing the absorbance at 620 nm of the resulting filtrate to unfiltered, centrifuged solutions.

Enzyme/Detergent Effects

The effects of SDS (sodium dodecylsulfate) on known enzymes was determined since SDS-protein complexes can result in loss of biological activity due to cooperative denaturation induced by SDS. The effect of different SDS concentrations (0.01, 0.05, .01, 0.5, and 1.0 wt%) on Genencor 150L and chymotrypsin activity on azure bound substrates was studied under assay conditions. The effects of SDS were also determined by pre-incubating the enzyme in the presence of detergent for one, 3, and 5 hours in Tris buffer at 37°C.

Preparation of Clarified Digester Sludge for Assay

Samples were obtained from a stable 2-liter digester fed MSW meal prepared from pellets procured from Thief River Falls, MN, by comminution in a Wiley knife mill fitted with a 2 mm rejection screen. Prior to analysis the samples were clarified by centrifugation and filtration with 0.22 micron Acrodisc filters. Concentrated digester supernatant was also examined and was prepared by ultrafiltration with Amicon PM 10 membranes. All assays were performed at 37°C and pH 7 to 7.5 to simulate the condition of the digester.

Digester Sludge Extractions

Digester sludge samples from a semi-continuous stirred tank reactor (CSTR) anaerobic reactor fed milled MSW, with a retention time of 14 d were collected under anaerobic conditions and transported to an anaerobic chamber in which the detergent extractions and enzyme analysis took place. Stock solutions (10%) of detergents were made and pre-reduced 24 h in the chamber. The extraction procedure consisted of agitation using a Fisher model 346

rotator at 37°C in the presence of the detergent for 4 h. The particulate material was then removed by centrifugation in 1.5 mL microcentrifuge tubes in a model Centra 4 desktop International (IEC) centrifuge at 10,000 x G for 20 minutes.

RESULTS

Supernatant Enzyme Activity

The analysis of free enzymes in clarified digester sludge must employ methods which do not depend on the production or destruction of an ultraviolet chromophore due to the very high UV background (e.g., 2.1 optical density units at 280 nm). Assay methods were chosen which detect the liberation of dye from appropriate dye-bound polymers or substrates. The yellow (p-nitrophenolate anion absorbing maximally at 420 nm) and blue (orcein and Remazol-brilliant Blue R or azure absorbing at 620 nm) color producing dyes showed adequate detectability in the presence of digester supernatant. Alternatively, and where dye-bound polymers were not commercially available, methods of polysaccharidase detection by analysis of the reducing sugars released using dinitrosalicylic acid (DNS) at 540 nm following incubation with substrate were also useful. Here, antibiotics were added to the incubation mixture to ensure reducing sugar loss via metabolism did not occur.

Samples were removed from a stable 2-liter digester fed MSW meal from Thief River Falls, MN. and treated as indicated in Material and Methods. Table I summarizes the results of successful assay methods on digester supernatants. These data show that clarified digester sludge contains very little detectable enzyme activity. Only alpha-amylase and general protease are within the limits of detection on 5x concentrated samples.

The digester supernatant was found not to inactivate the test enzymes examined (see Table I). All test hydrolytic enzymes chosen for evaluation of potential inhibiting effects elicited by digester supernatant were known to posses pH and temperature optima near those of the digester (e.g., pH 7 and 37°C). Acidothermus cellulolyticus enzymes were chosen because of broad pH and temperature optima. The Trichoderma cellulases normally used as a source of cellulase activities are virtually inactive at pH 7.

Dye Adsorption to Membranes

A filtration/adsorption study revealed that when Tris buffer alone is used to suspend the dye, the levels of adsorption can be quite severe. The order of binding of native dye to membrane according to membrane type is Nylon 66 > cellulose acetate/cellulose nitrate composite (Millipore HA) > polysulfone (Acrodisk) > PTFE (Millipore FH) = cellulose acetate (Millipore EG) > glass fiber (Millipore AP). The level of removal of dye is 99%, 68%, 59%, 13%, 13%, and 7% for the membrane types listed above, respectively. When enzymatically liberated dye is examined for membrane binding loss, lower levels of adsorption are observed. Here 5% and 7% of the original dye was lost on the glass fiber and cellulose acetate membranes, respectively.

When 2% ethylene glycol was added to the post-incubation mixture of enzyme released dye, subsequent filtration with cellulose acetate filters

showed no loss in dye above experimental error. This procedure will be well suited to rapid sample handling, as the cellulose acetate filters are available in a pre-assembled cassette from Millipore.

Dye/Detergent Extinction Coefficient Effect

A series of non-ionic and ionic detergents were examined for the ability to prevent binding of the released dye from the filter membrane. We examined the potential effect of the detergents on the absorbtivity of the dye in the visible spectrum, as an electronic conjugation induced by dye-dye association or dye-detergent complexes would be expected to increase the absorbance of the dye at the assay wavelength (620 nm). This condition would confuse the enzyme assay because the extinction coefficient of the dye in the presence of detergent would be different from that of native dye. Upon examination we found that an increase in molar extinction does indeed occur and that the order of effect is Brij > DOC (deoxycholate) > SDS (sodium dodecyl sulfate) > ethylene glycol. The non-ionic detergent, Brij, produced a 10% increase in absorbance and ethylene glycol produced no measurable effect.

Standardization of Dye-bound Assays with Classical Assays

Standard curves of enzyme activity were determined for several commercially available enzyme preparations using our developed protocols and azure substrates. The enzyme kinetics of the commercial preparations (Sigma and Genencor) are shown in Figures 1-4. A linear activity region was determined for all enzymatic activity protocols. Azure-linked substrate activity values will be referred to here as "apparent" units of activity.

Detergent Extraction of Activity from Digester Sludge

In these studies, most enzymatic assay protocols rely on the enzymes reacting with an insoluble substrate for activity measurement. This requires free enzymes and therefore assay protocols which dislodge the tightly bound enzymes from their present substrates in the sludge are needed. Several detergents were analyzed for their ability to solubilize enzymes that are bound to substrate or that may be tightly cell membrane associated. These detergents included SDS, Triton X-155, and Zwittergent detergents at varying concentrations tested using the azure bound substrates and the p-nitrophenol linked substrates (see Figures 5-8). The results showed that when digester sludge was extracted with Zwittergent detergent at low concentrations (0.01 to 0.05%) the apparent azure bound substrate activity increases with maximum apparent activity achieved when a concentration of 0.1% is reached (see Figure 7). Similar results were observed with beta-glucosidase activity which increased 147 times over control levels with the 0.1% Zwittergent extraction (data not shown). When Triton X-155 extractions were tested for apparent azure bound activity, a higher concentration of 1.0% was required to achieve maximum activity with hide powder azure (see Figure 8). The Triton X-155 extractions did not significantly increase the apparent activity on either amylose azure or of cellulose azure. SDS extractions of sludge showed similar results to the Triton X-155 extractions, however a lower concentration (0.1%) of SDS produced the maximum level of apparent hide powder activity (see Figure 6). The SDS extractions did not significantly increase the amylose azure activity but did, at the 0.05% level, increase the beta-glucosidase activity 119 times higher than control levels shown in Figure 5. No significant

enhancement of cellulose azure activity was observed with any of the detergent extractions that were tested.

Extractions of 0.1% SDS, 1.0% Triton X-155, and 0.1% Zwittergent detergents were compared for their ability to extract endo-beta-1,4 glucanase (CMCase) activity from anaerobic sludge. All detergents gave significantly higher levels of activities over control levels (see Table II). The 0.1% SDS extractions gave the highest levels of endo-glucanase activities when compared to Triton and Zwittergent extracts.

The effect of aerobic incubation of the assay was also compared for the different detergent extractions. There was not a significant difference between aerobic and anaerobic incubation of SDS extractions or between aerobic and anaerobic incubation of Triton X-155 extractions. A significant difference was found for aerobic incubation of the Zwittergent extraction with the aerobic levels being higher than the anaerobic activities.

Potential Detergent Inactivation of Test Enzymes

Since SDS is known for its ability to denature proteins under various conditions, we tested known enzymes to determine if a loss of activity occurred at concentrations of SDS used to extract activity from sludge. When alpha-chymotrypsin was tested with low levels of SDS (0.01%), an apparent enhancement of activity was noted. A concentration of 0.1% SDS slightly decreased the level of activity and levels of greater than 2% caused a significant loss of activity against hide powder azure. The effect of SDS on Genencor 150L cellulase activity was tested by adding SDS to the enzyme-substrate mixture and by pre-incubation under extraction conditions. No significant change in activity was noted due to the addition of SDS (0.1-1.0%) to the reaction mixture. A significant difference between the 1.0% and control extracts was observed with an enhancement of activity being responsible for the difference. Also, there was not a significant difference between 0.1% and 0.01% SDS when compared to control extracts.

DISCUSSION

The first stage of this project dealt with the problem of developing, or adapting, enzymes assays suitable for application to the particulate-free portion of the digester sludge. Apart from a few classical assays, such as the cellulase activity assays, assay protocols based on the liberation of dye from dye-substrate complexes proved well suited to digester supernatant samples. Using these methods, alpha-amylase and protease activities were detected in digester supernatant.

An examination was then made of the application of dye-bound substrates, such as hide powder azure, starch azure, amylose azure, and others, to the problem of determining available enzyme activity in the particulate fraction of the digester sludge. In using the dye-release assays with particulate samples we examined the following potential problems: loss of enzymatically released dye (in the incubation mixture) on the sludge particulates and loss of released dye on the filter membrane used to separate the unhydrolyzed dye-substrate from the free dye which is then measured spectrophotometrically. These concerns were reasonable in view of the chemical nature of the dye

itself, where both hydrophobic and hydrophilic binding interactions with a wide variety of materials is likely. Further studies at first indicated that several non-ionic detergents (Brij, Triton, etc) seemed effective in recovering dye lost from the incubation mixture on sludge particulates. It appears now that the extinction coefficient enhancement effect may have produced this apparent 10% recovery of dye from the sludge. Indeed, there may be little or no dye lost on the sludge particulates at all. This second cause of non-specific dye loss from the assay system, dye loss on filter membranes, was relieved by the discovery of weakly-adsorbing filter membranes.

The higher measured apparent activities found in the detergent extracted sludge over control levels indicate that the low previous activity measurements may be due to the tight binding of the enzymes to the particulate substrates in the sludge, or that the hydrolytic enzymes are tightly cell membrane associated. In either case dissociation of the enzyme from the particulate fraction in sludge was necessary for accurate measurement of the hydrolytic activity in the digester. The use of detergent extractions of sludge to measure the hydrolytic activity also eliminates the problems of Remazolbrillant blue binding to particulates in the reaction mixture and reduces the amount of dye-binding to the cellulose acetate filters when the reaction mixture is filtered. However, questions regarding the effect of these detergents on enzyme tertiary structure (and its ability to maintain activity in the presence of detergent) needed to be addressed. The detergents used in this study are commonly employed for the extraction of active enzymes from cell membranes [Gonenne and Ernst 1978; Hjelmeland et al 1979; Womack et al 1983] and are expected to leave many enzymes fully active after exposure. Studies designed to test possible loss of cellulase (fungal), chymotrypsin and alpha-amylase activities following exposure to solutions of SDS varying from 0.1 to 1.0% for one to 12 hours at 37°C, found no significant activity loss (data not shown). The effect of SDS on the digestibility of the hide powder substrate used for protease assays was also examined and found to be undetectable.

Upon examination of the overall activity values found for hydrolytic enzymes in digester supernatant and sludge, the relatively low titers of enzymes becomes a striking issue. Although the breadth of activities expected from a bacterial cell consortia of fairly high density were found (e.g., cellulase, protease, amylase), the activity levels were not high. For example, in biochemical depolymerization of woody biomass cellulase enzyme levels of highly active fungal enzymes are normally used at the 20 - 80 U/gram cellulose level. This loading has been shown to achieve complete conversion of cellulose in 2 to 10 days depending upon the level of pretreatment of the sample. Clearly, at the very low levels of cellulase activity found in digester sludges so far, cellulase augmentation would be highly beneficial. To date, however, only cellulase pretreatment of MSW feed prior to anaerobic digestion and in-digester augmentation of lipases (Higgins and Swartzbaugh, 1985) has been attempted. In contrast, the agro-industry has utilized cellulase pretreatments of complex feedstocks for enhanced anaerobic digestion for several decades [Labat et al 1984 and many others].

Microbiologically, however, the reason for this apparent inherent inability of microbial consortia obtained from large scale municipal installations to produce adequate quantities of free-cellulases remains a key impediment to optimal methane production rates and yields.

FUTURE DIRECTION

Now that a reliable set of assays for hydrolytic enzymes is available, the application of enzyme augmentation in small scale digesters fed MSW, with continuous measurement of enzyme activities, is an important direction to investigate. Also valuable would be the monitoring of samples from large scale anaerobic digesters fed MSW and soil samples from municipal land fill sites.

REFERENCES

Bergmeyer, H.U. 1974. Methods of Enzymatic Analysis. Vol.II. Academic Press, New York.

Bernfeld, P. 1955. Methods Enzymol. 1:149.

Fernley, H.N. 1963. Biochem. J. 87:90-95.

Ghose, T.K. 1987. Pure and Appl. Chem., 59:2

Gonenne, A., and Ernst, R. 1978. Anal. Biochem. 87:28-38.

Khan, A.W., and van den Berg, L. 1981. Biotech. Bioeng. Symp. 11:325-331.

Higgins, G., and Swartzbaugh, J.T. 1985. Energy from Biomass and Wastes IX. Institute of Gas Technology, Chicago, IL. pp. 791-810.

Himmel, M.E., Tucker, M.P., Oh, K.K., Rivard, C.J., and Grohmann, K. 1986. Biotech. Bioeng. Symp. 17:413-423.

Hjelmeland, L.M., Nebert, D., and Chrambach. 1979. Anal. Biochem. 95:201-208.

Labat, M., Garcia, J.L., Meyer, F., and Deschamps, F. 1984. Biotechnology Letters. 6:379-384.

Mandels, M., Andreotti, R., and Roche, C., 1976. Biotechnol. Bioeng. Symp. 6: 17.

Miller, G.L. 1959. Anal. Chem., 31:426.

Mohagheghi, A., Grohmann, K., Himmel, M.E., Leighton, L., and Updegraff, D. 1986. Int. J. Systematic Bacteriology. 36:435-443.

Reilly, P.J. 1981. in Trends in the Biological Fermentations for Fuels and Chemicals. Hollaender, A. ed., Plenum Press, New York, pp. 111-130.

Rinderknecht, H., Wilding, P., and Haverback, B.J. 1967. Experientia 23:805.

Sternberg, D., Vijaykumar, P., and Reese, E.T. 1977. Can. J. Microbiol., 23, 139.

Tate, R.L., and Klein, D.A. 1985. Soil Reclamation Processes. Marcel Dekker, Inc, New York. pp. 141-172.

Womack, M.D., Kendall, D.A., and Macdonald, R.C. 1983. Biochim. Biophys. Acta. 733:210-215.

Wood, T.M. 1971. Biochem. J. 121:353.

Yeoh, H.H., Wong, F.M., and Lim, G. 1986. Mycologia. 78:298-300.

TABLE I

ENZYME ACTIVITIES IN DIGESTER SUPERNATANTS

ASSAY SUBSTRATE	SOURCE OF TEST ENZYME	SUPERNATANT ACTIVITY (NEAT)	SUPERNATANT ACTIVITY 5x CONC.	INHIBITION OF KNOWN ENZYMES BY SLUDGE
filter paper	Acidothermus	-0-	-0-	no
CMC	Acidothermus	-0-	-0-	no
oat xylan	Acidothermus	-0-	-0-	no
starch-azure	porcine pancr.	-0-	-0-	no
amylose-azure	porcine pancr.	-0-	**	no
keratin-azure	none	-0-	-0-	NT
pNPbetaglucoside	almonds	-0-	-0-	no
	Acidothermus	-	-	no
pNPalphaglucos	brewer yeast	-0-	-0-	no
pNPglucuronide	none	-0-	-0-	NT
pNPbetagalacto.	none	-0-	-0-	NT
pNPxyloside	Acidothermus	-0-	-0-	no
pNP-N-acetyl-glucosamidase	none	-0-	-0-	NT
pNPlactopyran.	none	-0-	-0-	NT
pNPpalmitate	Pseudomonas	-0-	-0-	no
total protease [casein clearing]	trypsin	-0-	***	no
total protease [hide powder-azure]	trypsin	-0-	***	no
total protease [azo-casein]	trypsin	-0-	-0-	no

**Equivalent to 0.10 unit alpha-amylase/mL sludge.
***Trace activity found, but less than 2 ug trypsin equivalent/mL sludge.

Table II

MAXIMUM APPARENT* ACTIVITIES OBTAINED FROM DIGESTER SLUDGE

Substrate	Activity (units/mL sludge) Control	Triton	SDS	Zwittergent
hide powder-azure[a]	0.000	0.049	0.034	0.023
amylose-azure[b]	0.007	0.020	0.035	0.047
starch-azure[b]	0.000	NT	0.000	0.000
cellulose-azure[c]	0.000	<0.010	0.000	<0.010
CMC[d]	0.000	98.710	247.910	146.700
pNP-beta-glucoside[e]	0.003	NT	0.045	0.029
pNP-alpha-glucoside[e]	0.001	NT	0.016	NT

* a. From standard curve where one unit equals that amount of enzyme required to hydrolyze 1.0 umole of BTEE per min at pH 7.8 and 25°C.

b. From standard curve where one unit equals that amount of enzyme required to liberate 1.0 umole of maltose from starch per 3 minutes at pH 6.9 and 30°C.

c. From standard curve where one unit is defined as one filter paper unit (see text).

d. Defined as one unit equals that amount of enzyme required to liberate one ug glucose from CMC per 30 min at pH 7.5 and 37°C.

e. Defined as one unit equals that amount of enzyme required to produce of one p-nitrophenolate anion per minute at pH 7.5 and 37°C.

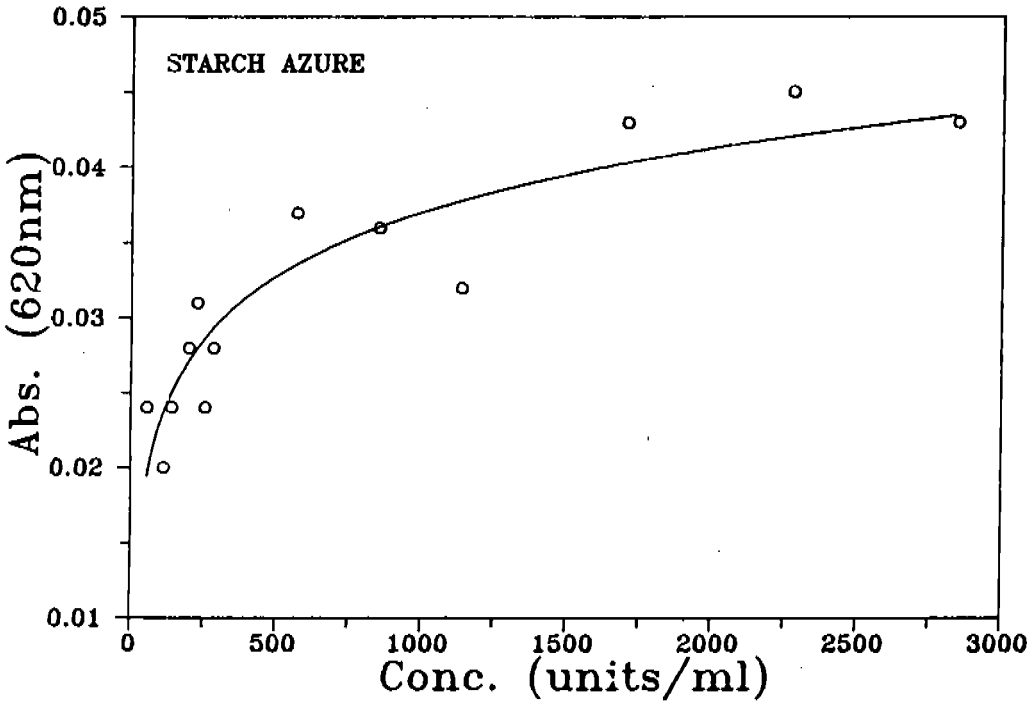


Figure 1. Standard curve of alpha-amylase using starch azure substrate, 4 h incubation at 37oC in 100 mM Tris pH 7.5. Units of enzyme activity taken from Sigma Chem.

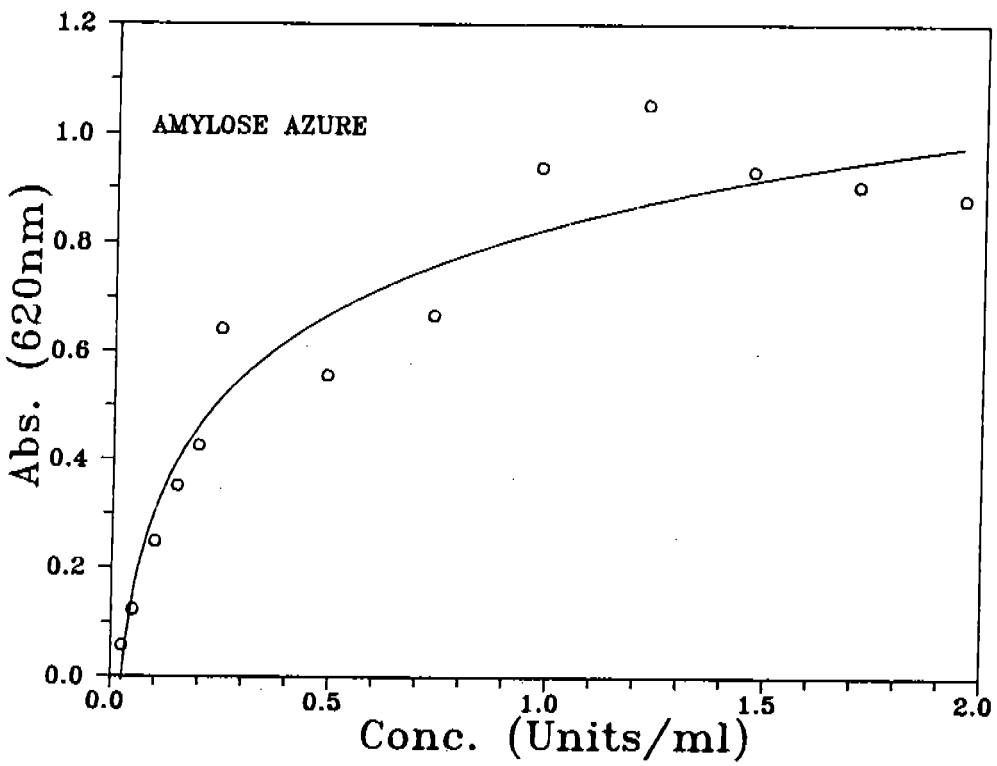


Figure 2. Standard curve of alpha-amylase using amylose azure substrate, 4 h incubation at 37oC in 100 mM Tris pH 7.5.

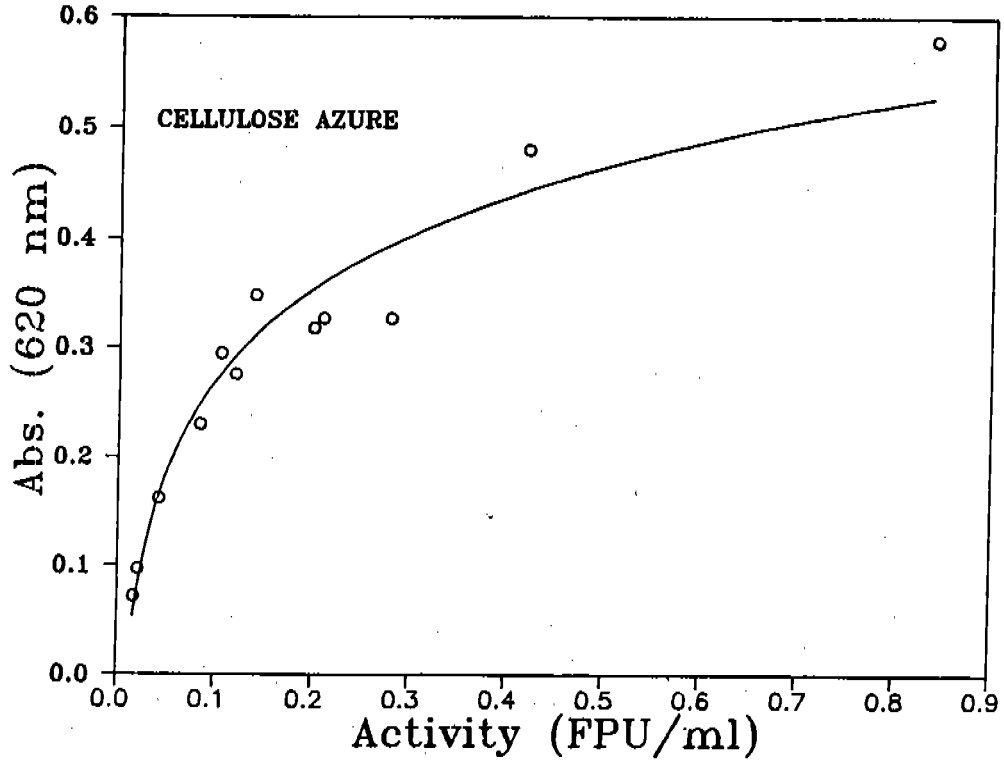


Figure 3. Standard curve of Genencor 150L cellulase using cellulose azure substrate, 4 h incubation at 37oC in 100 mM acetate pH 5.0. Units of filter paper activity were determined as described in text.

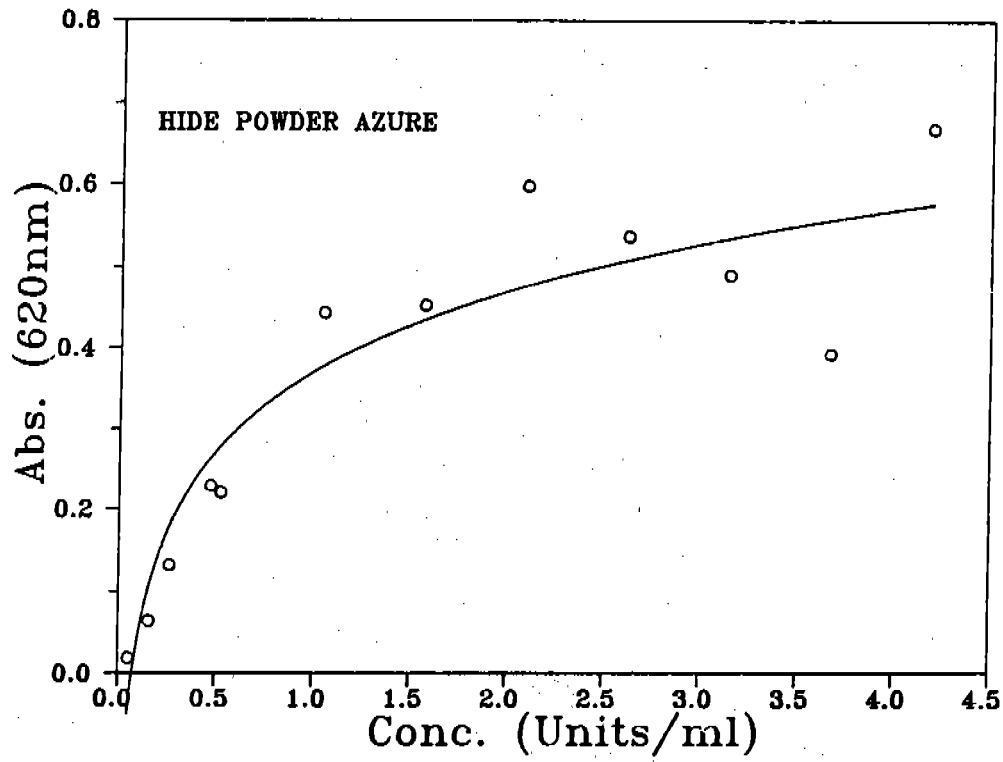


Figure 4. Standard curve of alpha-chymotrypsin using hide powder azure substrate, 4 h incubation at 37oC in 100 mM Tris pH 7.5. Units of enzyme activity taken from Sigma Chem.

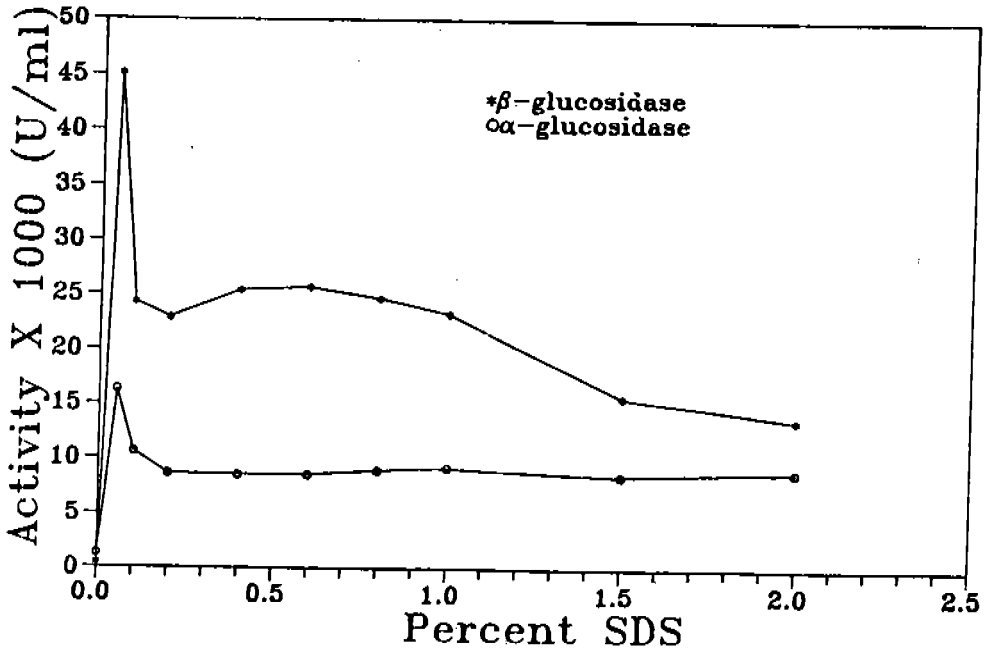


Figure 5. Effects of 4 h SDS extractions of digester sludge on recoverable alpha- and beta-glucosidase activity.

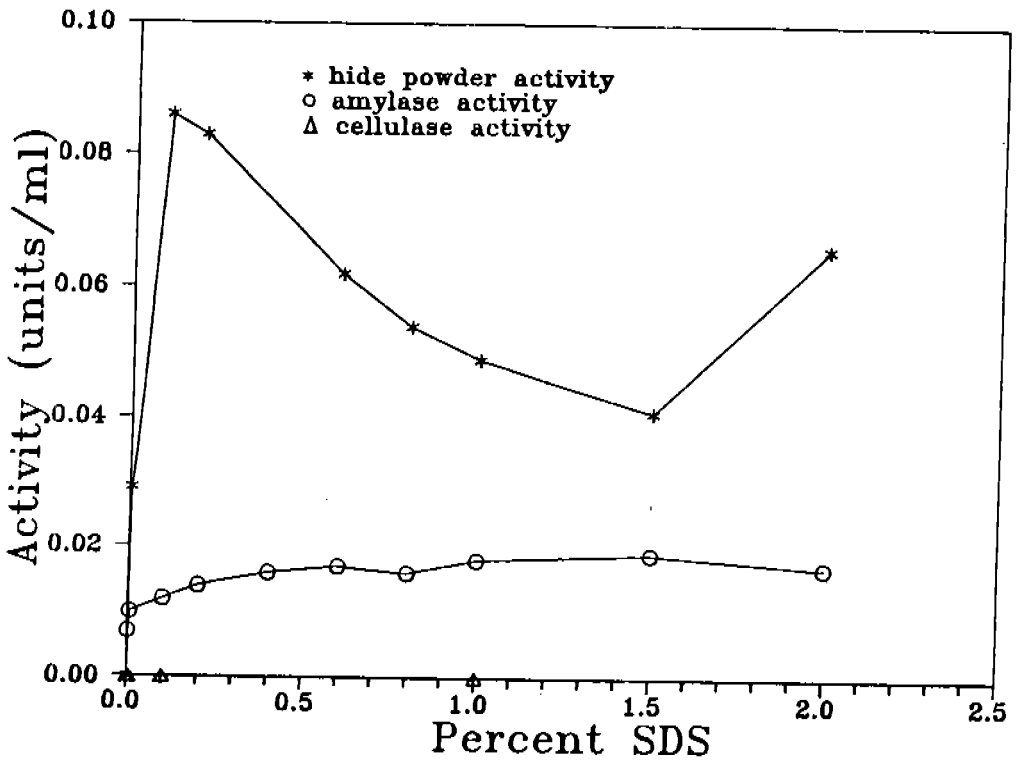


Figure 6. Apparent azure-linked activities from SDS extracted digester sludge.

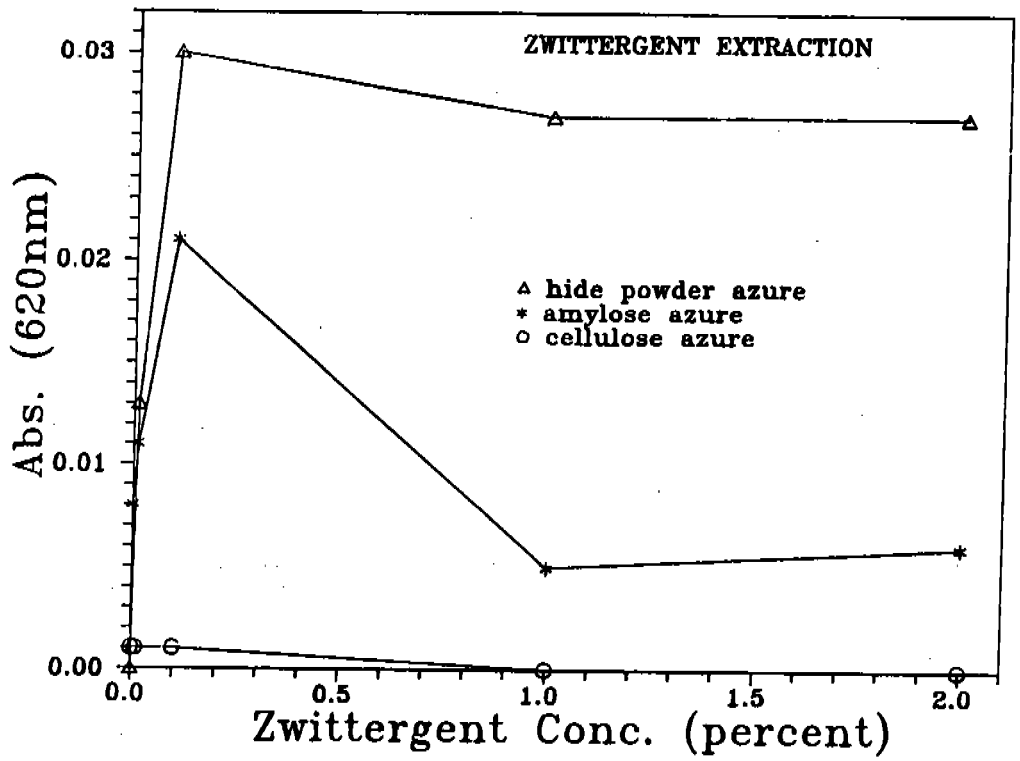


Figure 7. Apparent azure-linked activities from Zwittergent extracted digester sludge.

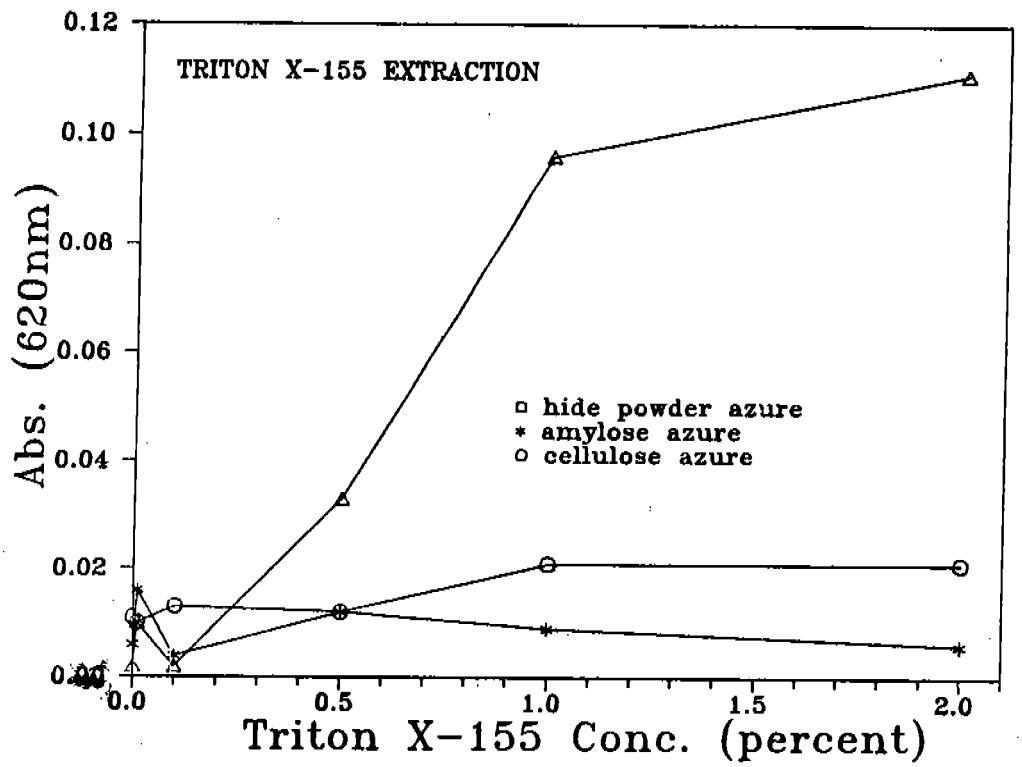


Figure 8. Apparent azure-linked activities in Triton X-155 extracted digester sludge.

PHOTOENHANCED ANAEROBIC DIGESTION OF ORGANIC ACIDS

P. Weaver and P.-C. Maness
Solar Energy Research Institute
Golden, Colorado 80401

ABSTRACT

The slow steps of the soluble reactions that occur in anaerobic digestion are the conversion of organic acids into H_2, CO_2, and acetate and the conversion of acetate into methane. Undoubtedly they are slow due to energetic constraints. Photosynthetic bacteria can utilize solar energy to generate ATP, which can then drive energetically unfavorable reactions. Under conditions of low intensity illumination, they readily produced H_2 from organic acids, including acetate. Equilibrium partial pressures of H_2 were well above the K_s for H_2 of methanogenic bacteria. The isolation of numerous strains of photosynthetic bacteria from actively methanogenic environments suggests that this may be a natural commensalism. Mixed cultures of photosynthetic bacteria with non-aceticlastic methanogenic enrichments exhibited large increases in methane production from acetate, propionate, butyrate, and lactate. The photosynthetic bacteria photoassimilate the organic acids to the level of endogenous sugars and sugar precursors, and then ferment the sugars to pyruvate in a subsequent dark reaction. In Rhodospirillum rubrum pyruvate is cleaved producing formate, which is a precursor to H_2.

PHOTOENHANCED ANAEROBIC DIGESTION OF ORGANIC ACIDS

INTRODUCTION

Analysis of batch anaerobic digestors (Zehnder et al., 1982) indicates that only 4% of the methane produced comes directly from the H_2 and CO_2 generated during fermentation (20% comes directly from fermentative acetic acid and 76% comes indirectly from other organic acids following obligate proton-reducing acetogenesis). Methane production from organic acids, including acetic acid, is slow compared to that from H_2 plus CO_2. The slow conversion of organic acids into methane precursors is readily understandable from energetic bases, since little chemical free energy becomes available. The reactions proceed in the direction of organic acid breakdown only because the H_2 product is scavenged to very low concentrations by methanogenic bacteria. With this necessary requirement for low steady-state levels of H_2, methane production is less than maximal. Average digester rates of about one volume of methane produced per equal volume of liquid per day (VVD) can be incresed to about 20-40 VVD when supplemental H_2 is provided (Bryant et al., 1968; Tracy and Ashare, 1981) and to 220 VVD when H_2 at high pressures (to effect mass transfer) is combined with cell recycle (Tracy and Ashare, 1981). If organic acids could be totally converted to H_2 and CO_2 at high rates (especially if polymer hydrolysis rates can also be improved), then CH_4 production rates should also be enhanced.

Photosynthetic bacteria, often found in natural environments with methanogenic bacteria, are unique in that they have the ability to absorb light energy, which can be used to drive otherwise energetically unfavorable reactions. The preferred substrates for photosynthetic bacteria are organic acids, which are assimilated into cell storage material during periods of illumination and then refermented into primarily H_2, CO_2 and acetate products during succeeding periods of darkness (Schultz and Weaver, 1982). Acetate can be further converted to H_2 plus CO_2 by light-dark cycles. Repetitive light-dark cycles thus provide the energy and the mechanism for rapidly removing organic acids and converting them into the optimal substrates for methanogenic bacteria, H_2 and CO_2 (Fig. 1).

Conversion of organic acids into H_2 and CO_2 by photosynthetic bacteria occurs under either of two conditions involving low intensity radiant (e.g., solar) energy inputs. The activity is induced either with very low intensity continuous illumination (as would occur at the bottom of a lagoon) or with repetitive short periods of sunlight followed by long periods of darkness (as would be perceived by a bacterium in a stirred tank reactor with a transparent cover). The common factor for both conditions is that photosynthetic and dark, fermentative metabolisms are simultaneously induced.

Since the organic acids are produced as free acids by fermentative

bacteria, the pH of a digester can rapidly drop leading to failure, especially at high loading rates. A mechanism to rapidly remove the organic acids as they are made will thus also increase digester stability.

RESULTS AND DISCUSSION

Methanogenic enrichments from an anaerobic digester were maintained on pre-reduced media (Weaver et al., 1975) containing bicarbonate, minerals, vitamins, 0.5 g/l yeast extract, and either propionate, butyrate or lactate as the fixed carbon source. About 30 pure strains of photosynthetic bacteria were screened on the same media for rates and steady state levels of H_2 production with exposure to low intensity (10 W/m^2) continuous incandescent light. Two strains of Rhodospirillum rubrum and one of Rhodopseudomonas viridis were selected for further study.

Rates of methane production in the enrichment cultures were slow and none of the cultures could produce detectable amounts of CH_4 when shifted to a medium with acetate as sole source of fixed carbon. When the Rps. viridis strain was inoculated into acetate medium with the methanogenic enrichment and exposed to low levels of light, methane formation was observed, although at slow rates. Addition of the photosynthetic bacterium and light to the butyrate and propionate enrichment cultures produced 7-fold and 10-fold increases in methane production, respectively. Interestingly, a smaller enhancement on methane production rates was observed even when cultures containing photosynthetic bacteria were incubated in continuous darkness. No increases were observed when heat-killed bacteria were added.

Elucidation of the mechanism by which photosynthetic bacteria convert organic acids into H_2 and CO_2 is complicated by the multiplicity of enzymes present that mediate the inducible H_2 metabolisms. At least four, and possibly six, distinct hydrogenase enzymes affect H_2 production and its partial pressures. Nitrogenase and CO-linked hydrogenase activities are not induced under the methanogenic conditions used, however.

In the case of R. rubrum, pyruvate is a precursor to H_2, probably through pyruvate-formate lyase and "formate-hydrogenlyase" activities. Dark rates of H_2 production were 440 μmol/min-g cells using formate and about 25% of that for pyruvate. A brief exposure of the cells to low intensity light caused about a two-fold stimulation of H_2 production from pyruvate, but not from formate. The addition of pyrophosphate to the cells caused a similar stimulation, indicating that ATP may be consumed in or regulating pyruvate metabolism. Chemical uncouplers of ATP synthesis abolished the stimulations. An equilibrium partial pressure of about 0.15 atmospheres H_2 was obtained in the cultures, although this varied considerably depending on the age of the cultures and the carbon substrate on which they were grown. Induction of an uptake hydrogenase with increased culture age on some carbon substrates probably functioned to re-consume some of the H_2 and lower the

steady state levels. Substrate oxidation linked to H_2 production activity was lost when cells were broken or converted into spheroplasts under strictly anaerobic conditions, making biochemical descriptions of the processes difficult.

FUTURE WORK

Since the dark, fermentative reactions of photosynthetic bacteria appear to be rate-limiting in the conversion of organic acids into H_2 and CO_2, a survey of about 200 additional strains has been initiated to identify those strains that ferment sugars in darkness faster, and therefore produce H_2 faster, while being less sensitive to H_2 partial pressures. Additional information is needed on the metabolic pathways employed by photosynthetic bacteria for H_2 production, how they are regulated, and their rate-limiting steps. Mutant strains are being isolated that are defective in different hydrogenase and terminal fermentative proteins in order to identify the protein's biochemical activity in whole cells. The potential for photosynthetic bacteria to act as obligate proton reducing acetogenic bacteria in continuous darkness will be pursued using strains that can be retrieved and identified after long-term incubation in digesters. Three different digester designs are being examined that will permit solar penetration.

REFERENCES

Bryant, M. P., McBride, B. C., and Wolfe, R. S. 1968. J. Bacteriol. 95:1118.

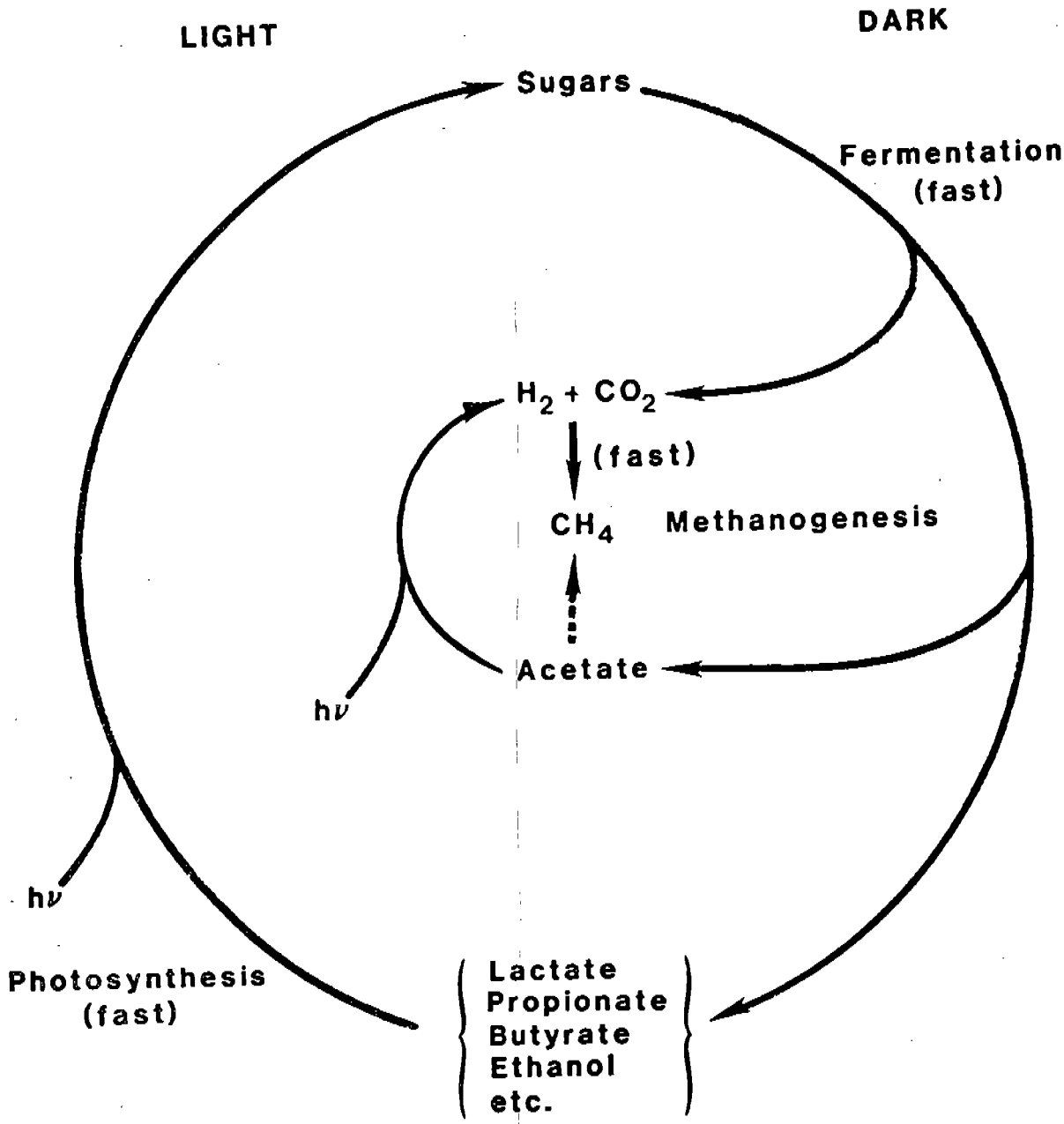
Schultz, J., and Weaver, P. 1982. J. Bacteriol. 149:181-90.

Tracy, C. A., and Ashare, E. 1981. Dynatech R/D Final Report on SERI Contract No. XB-9-8356-1. Report No. 2125. Cambridge Mass.

Weaver, P. F., Wall, J. D., and Gest, H. 1975. Arch. Microbiol. 105:207-16.

Zehnder, A. J. B., Ingvorsen, K., and Marti, T. 1982. Anaerobic Digestion 1981. D. E. Hughes, et al., eds., Elsevier, p. 45.

Fig. 1. Scheme for photoenhanced conversion of organic acids into methane.



EFFECTS OF HYDROGEN ON ACETATE DEGRADATION BY *METHANOSARCINA* SPECIES

DAVID R. BOONE* and ROBERT A. MAH

ENVIRONMENTAL AND OCCUPATIONAL HEALTH SCIENCES
SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF CALIFORNIA
LOS ANGELES, CA 90024

*Current address: Environmental Science and Engineering, Oregon Graduate Center, Beaverton, OR 97006-1999

ABSTRACT

Acetate and hydrogen are the major sources of methane in anaerobic digestion, acetate because it accounts for two-thirds of methane produced, and hydrogen also because its concentration controls many other reactions of anaerobic digestion. Hydrogen is an unusual intermediate because its concentration is extremely low (about 0.1 μM). Therefore it turns over very rapidly (perhaps hundreds of times per second) and has the potential to fluctuate rapidly in digestors. Substrate levels of hydrogen inhibit acetate degradation, but the effects of low hydrogen concentrations, such as occur in digestors, is not known and was the subject of this investigation. We grew strains of *Methanosarcina* on acetate, and maintained various concentrations of hydrogen during growth. We found that hydrogen pressure between 2 Pa and about 1000 Pa affected neither specific growth rate nor the rate of methanogenesis. Our data indicated that when hydrogen pressure is very low, *Methanosarcina* produce hydrogen during growth on acetate, but that the rate of hydrogen production is slow relative to the rate of acetate catabolism, and hydrogen production doesn't significantly affect the stoichiometry of acetate catabolism.

EFFECTS OF HYDROGEN ON ACETATE DEGRADATION BY *METHANOSARCINA* SPECIES

INTRODUCTION

H_2 pressure controls many reactions in anaerobic digestors by its effects on several metabolic groups of bacteria (3), including aceticlastic methanogens (7). Because acetate is quantitatively the most important precursor of methane in anaerobic digestors, we examined acetate degradation and how it is affected by H_2 pressure.

As late as 1978 the ability of *Methanosarcina* species to catabolize acetate as sole substrate was controversial. In 1975 Zeikus et al. (18) showed that, when cultures were incubated with radio-labelled acetate, tiny amounts of labelled methane were produced and the amount was dependent on the amount of H_2 added to cultures. These workers concluded that methanogenesis from acetate in nature required H_2. They went on to speculate that methanogens might reduce both carbons of acetate, thus producing two methane molecules in the presence of H_2. As late as 1977, in his review article on methanogens (17), Zeikus indicates that *Methanosarcina barkeri* can use only H_2-CO_2 or methanol (but not acetate) as sole catabolic substrate. In 1978, it was shown that *M. barkeri* 227 could indeed grow in medium with acetate as the sole organic addition and the sole catabolic substrate (14). Studies in other laboratories often failed to demonstrate acetate catabolism by *Methanosarcina* species; in experiments performed for this contract we showed that this inability to grow on acetate was probably due to the way the inocula were grown. When H_2-grown cultures are transferred to medium with acetate as sole catabolic substrate, often no growth occurs (7). When growth does occur, it is only after a lag period of a month or more. When such H_2-grown cultures are transferred to medium H_2 as substrate, growth begins without a lag. When medium with substrate quantities of both H_2 and acetate is inoculated, methanogenesis from acetate is postponed until H_2 utilization is complete (7). As soon as H_2 is exhausted, methanogenesis resumes with acetate as catabolic substrate, resulting in a bi-phasic growth pattern (7). These strange effects of H_2 on acetate degradation by *M. barkeri* 227 led us to examine this phenomenon more closely. Because H_2 turns over rapidly in digestors, and because its *in situ* concentration may vary greatly either temporally or spatially (2,3,5), we chose to investigate whether H_2 concentration affects *M. barkeri* in a way which could have implications on acetate degradation in anaerobic digestors. These facts led us to investigate why H_2-grown inocula of *M. barkeri* could not grow on acetate whereas cells in culture medium with both substrates could switch from H_2 to acetate without a lag. (Portions of these results were presented previously [5,6].)

EXPERIMENTAL DESCRIPTION

M. barkeri 227, *M. barkeri* MS, *M. barkeri* UBS, and *M. mazei* S-6 were from our culture collection; *Desulfovibrio* sp. strain G11, *Methanospirillum hungatei* JF1, *Syntrophomonas wolfei* co-cultures were a gift from Marvin P. Bryant. Pure cultures were maintained as previously described (6); unless stated otherwise, inocula of aceticlastic methanogens were grown to late-exponential phase in medium with 50 mM acetate as catabolic substrate. "H_2-

grown inocula" were grown in medium with H_2 and CO_2 as the sole catabolic substrate. Transfer volumes were 2.5% (vol/vol), except that 5% (vol/vol) was used for axenic cultures of *Methanosarcina mazei* and 50% (vol/vol) for *S. wolfei* co-cultures. All cultures were incubated statically at 37°C, except certain cultures grown on added H_2, which were grown on a shaker at 37°C. All experiments were done in triplicate, and reported values of methane produced are the geometric means of the replicates. Reported experiments always had only small variations among replicates. Experiments were done with alpha medium (6). The culture techniques of Hungate (8) as modified by Balch et al. (1) were used throughout these studies. Both optical density and methane production were used as a measure of specific growth rate. Because *Methanosarcina* species, especially *M. mazei*, vary greatly in size and shape, they do not strictly follow Beer-Lambert absorbance relationships. In addition, *M. mazei* aggregates and cysts are so large that they cannot be kept in suspension during measurements of optical density. Thus, we rely heavily on product formation for measurement of growth; unless indicated otherwise, growth rates are calculated from methane production after addition of the methane produced by the inoculum (13). We also determined that methane production was not uncoupled from growth. Continuous-culture experiments were performed by using a BioFlo fermentor (New Brunswick Scientific Co., Inc., Edison, NJ). The vessel had a 350-ml working volume. The empty, sterile fermentor was flushed with O_2-free nitrogen gas and inoculated with cultures of *M. barkeri* 227 (300 ml) and *Desulfovibrio* sp. strain G11 (50 ml). Medium was pumped into the vessel at a rate of 1.59 ml h^{-1}, giving a 9.2-day retention time. The continuous-culture fermentor was operated until pH, sulfide, acetate concentration, and gas production were stable for seven consecutive days and then measured those parameters for five additional days. Reported values are averages for the last five days.

Gases were analyzed by gas chromatography and detected by thermal conductivity (CH_4 and CO_2) or by vaporization of HgO (H_2). Acetate and butyrate were quantified by gas chromatography of the free acids. Sulfide was determined by using the methylene blue method (15).

RESULTS

Aceticlastic growth of *Methanosarcina* with altered H_2 pressure. The effect of elevated H_2 concentration on acetate catabolism by methanogens was examined. Although very high H_2 pressures had been tested (7), nothing was known about the possible effect of smaller increases. We added small amounts of H_2 (*ca.* 100 Pa) to the headspace of growing, aceticlastic cultures of *M. barkeri* or *M. mazei*. We incubated the cultures with shaking, and monitored the concentration of H_2 (Fig. 1). Cells took up H_2, as shown by a decrease in its pressure, which rapidly approached the pressure in controls without added H_2 (20 to 30 Pa). In the same experiments we removed H_2 from other cultures by flushing the headspace with O_2- and H_2-free gas. In these cultures the cells formed H_2 which accumulated again to pressures similar to those of control cultures. The quantity of H_2 produced or consumed in these reactions was always very small compared to the rate of methanogenesis which was occurring simultaneously. This fact, and the slow, asymptotic approach of H_2 pressure to the level of controls, suggests that H_2 production or uptake in these systems is not related to catabolism but

may be simply an equilibrium process between external H_2 and electron carriers within the cells.

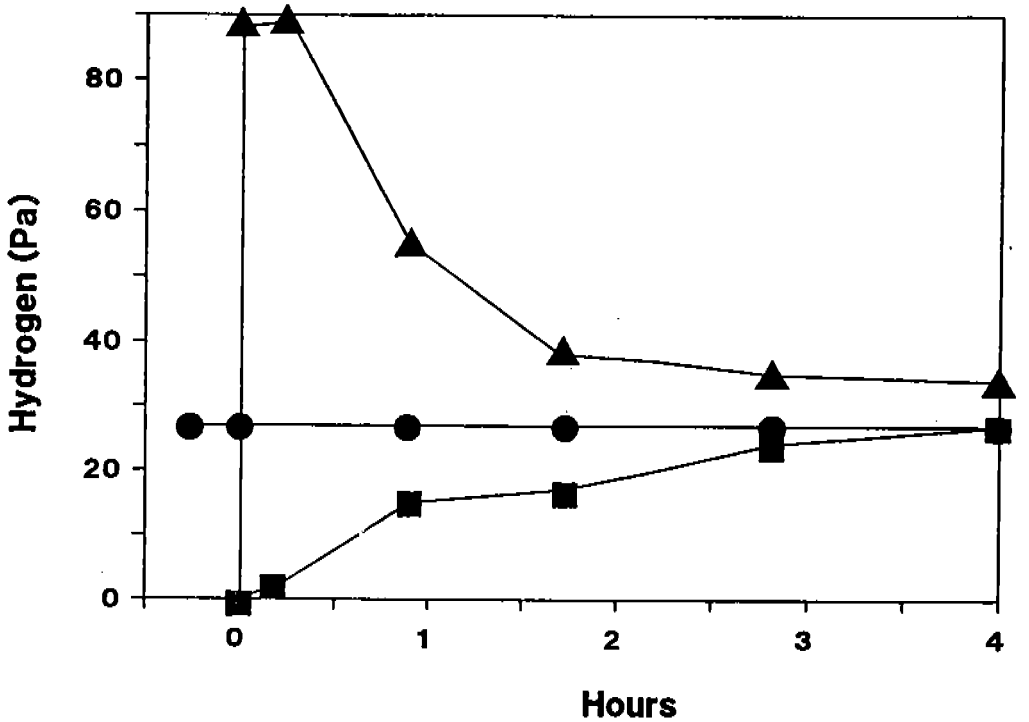


Figure 1. H_2 metabolism by aceticlastic *M. barkeri* cultures. Cultures were grown on acetate, and hydrogen was added to some cultures (▲) and flushed from others (■).

Use of *Desulfovibrio* sp. strain G11 to maintain H_2 concentration. To determine whether continued maintenance of low H_2 pressure would affect acetate degradation we grew *M. barkeri* in co-culture with a desulfovibrio. By using medium with 5 mM sulfate and by varying the initial concentration of lactate, we could elicit H_2 production or H_2 utilization by the desulfovibrio (6). In batch co-cultures of this desulfovibrio and *M. barkeri* 227 we maintained partial pressures of H_2 during exponential acetate degradation at various levels between 5 and 1000 Pa. This range is much greater than would be expected in anaerobic digestors, yet methanogenesis was unaffected.

In addition, we showed that *M. barkeri* probably doesn't catabolize H_2 at the pressures which are found in digestors. We tested the ability of *M. barkeri* 227 to catabolize H_2 at low concentrations by using *S. wolfei* as an H_2 source. *S. wolfei* was chosen because it produces H_2 from butyrate only when H_2 pressure is low. Ruminal methanosarcinae remove H_2 at the low pressure produced during butyrate catabolism by a butyrate-oxidizing anaerobe (probably a strain of *S. wolfei*)(10). We inoculated *S. wolfei*, *Desulfovibrio* sp. strain G11, and *M. barkeri* 227 into media with 20 mM butyrate. In such medium without sulfate, *Desulfovibrio* sp. strain G11 is unable to catabolize H_2. *S. wolfei* can catabolize butyrate only when H_2 is removed by another organism, and even then it can produce only a very small pressure of H_2 (11). Thus, growth and methanogenesis should proceed only if the methanosarcina can catabolize H_2 at very low pressures. Methane

production from such a culture is minimal (6). Addition of small amounts of sulfate increased methane production, probably because the desulfovibrio was able to use some H_2, allowing *S. wolfei* to produce some acetate from butyrate. This acetate could be used as a substrate for the methanogen to produce some excess methane. The methane produced is much less that would be expected stoichiometrically from the complete catabolism of butyrate, and most of the butyrate remained in these cultures at the end of the experiment (6). If, after 17 days incubation, excess sulfate was added to the cultures which initially contained limiting sulfate, and methanogenesis was greatly stimulated. This probably reflects the ability of the sulfate-reducer to remove H_2, which allowed the butyrate oxidizer to produce acetate, which allowed the methanosarcina to produce methane from acetate. If excess sulfate was present from the start methanogenesis occurred much earlier. All of these data are consistent with the inability of *M. barkeri* 227 to catabolize H_2 produced at low pressures from butyrate. This finding can be extended to imply that *M. barkeri* cannot catabolize significant quantities of H_2 in digestors.

H_2 production by aceticlastic cultures of *Methanosarcina* sp. The above experiment confirmed reports from other laboratories that other methanosarcinae (9) produce H_2 when H_2 pressure is extremely low. In these experiments, H_2 was removed from the head-space atmosphere by flushing with gas. As acetate degradation by these cultures continues, H_2 concentration increases to the levels present before its removal (9). In such experiments, a very small amount of produced H_2 brings the H_2 pressure back to this level. We then tested whether maintenance of low H_2 pressure would affect methanogenesis from acetate, or, if H_2 production was continuous and rapid, the stoichiometry of aceticlastic methanogenesis. If other microbes metabolize H_2 much more rapidly than the methanosarcinae, they may maintain H_2 pressure at a level low enough to stimulate constant H_2 production by methanosarcinae. Under these conditions, H_2 production could significantly affect the stoichiometry of acetate catabolism by stimulating acetate oxidation to CO_2. In fact, Phelps et al. (12) reported that another methanosarcina oxidized a significant fraction of acetate when co-cultured with an H_2-utilizing desulfovibrio. We developed a co-culture of *M. barkeri* 227 and *Desulfovibrio* sp. strain G11 in a continuous culture (6). We manipulated substrates to give an excess of sulfate with which the desulfovibrio maintains a pressure of H_2 of 2.2 Pa. We quantified methane in order to determine whether there was any significant diversion of electrons from the normal course of methane production. Under these conditions, total methane production from acetate is 93% of expected, based on stoichiometry. This value does not take into account that some acetate is used for anabolic purposes, and is well within the normal methane recoveries, which are 90 to 95% in batch studies (unpublished data from this laboratory). This 93% also includes acetate produced stoichiometrically from lactate. Thus, even partial pressures of H_2 as low as 2.2 Pa do not stimulate significant H_2 production from *M. barkeri* 227.

Effect of inoculum condition on ability to degrade acetate. Until recently it was not clear that *Methanosarcina* species could produce methane from acetate as sole substrate. The data from Mah's laboratory could not be documented in many other laboratories. In this study, we have shown that the reason for this inability to duplicate experiments was related to the state of inocula for experiments. We show that H_2-grown inocula, stored or incubated for a time after substrate is depleted, fail to grow or produce methane when inoculated into media with acetate as sole catabolic substrate,

and that addition of very small quantities of H_2 enabled these cultures to begin growth (6).

DISCUSSION

When viewed as a homogeneous system, well-functioning anaerobic digestors have H_2 partial pressures too low (below 10^{-4} atmospheres) to affect acetate degradation by *Methanosarcina* species. However, microenvironments with higher concentrations of H_2 may exist in digestors, and in these locations H_2 may inhibit acetate degradation. However, these microenvironments would probably be associated with biodegradable particulate matter, and elevated H_2 concentration in such locations would more likely have an effect on fermentative bacteria.

H_2 concentration would be expected to increase immediately after substrate addition, such as batch-feeding of digestors. This elevated H_2 concentration may be high enough to inhibit *Methanosarcina* species, but the duration of exposure would be brief. Our experiments show that when H_2 returns to low levels aceticlastic methanogenesis resumes quickly. Thus, aceticlastic methanogenesis in digestors appears to be independent of H_2 pressure in well-functioning digestors. In digestors which are inhibited, H_2 may accumulate to concentrations which inhibit aceticlastic methanogenesis. If only H_2-oxidizing methanogens are inhibited, acetate degradation by *Methanosarcina* may be inhibited by accumulated H_2, and under these conditions *Methanosarcina* may catabolize H_2. However, it is likely that if environmental conditions in unstable digestors inhibit H_2-oxidizing methanogens, those conditions will also inhibit the aceticlastic methanogens.

ACKNOWLEDGEMENTS

We thank Indra Mathrani, Bhavana Likhananont, José Menaia, Jane Boone, and Gloria Maestrojuán for technical assistance. This work was supported by DOE a grant (33211402) from Argonne National Laboratory.

LITERATURE CITED

1. **Balch, W. E., and R. S. Wolfe.** 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM) dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl. Environ. Microbiol. 32:781-791.
2. **Boone, D. R.** 1984. Mixed-culture fermentor for simulating methanogenic digestors. Appl. Environ. Microbiol. 48:122-126.
3. **Boone, D. R.** 1985. Fermentation reactions of anaerobic digestion. *In* P. N. Cheremisinoff and R. P. Ouellette (eds.) Biotechnology; applications and research, pp. 41-51. Technomic Publishing Co., Inc., Lancaster, Pa.
4. **Boone, D. R.** 1985. Thermodynamics of catabolic reactions in the anaerobic digestor. In: A. A. Antonopoulos, ed., First symposium on

biotechnological advances in processing municipal wastes for fuels and chemicals. Academic Press.
5. **Boone, D. R., and R. A. Mah.** 1987. Effect of molecular hydrogen on acetate degradation by *Methanosarcina barkeri* 227. *In* A. T. Bull and G. Holt (eds.) Perspectives in biotechnology, pp. 111-121.
6. **Boone, D. R., J. A. G. F. Menaia, J. E. Boone, and R. A. Mah.** 1987. Effects of hydrogen pressure during growth and effects of pregrowth with hydrogen on acetate degradation by *Methanosarcina* species. Appl. Environ. Microbiol. 53:83-87.
7. **Ferguson, T. J., and R. A. Mah.** 1983. Effect of H_2-CO_2 on methanogenesis from acetate or methanol in *Methanosarcina* spp. Appl. Environ. Microbiol. 46:348-355.
8. **Hungate, R. E.** 1969. A roll tube method for cultivation of strict anaerobes, p. 117-132. *In* R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 3B. Academic Press, Inc., New York.
9. **Lovely, D. R., and J. G. Ferry.** 1985. Production and consumption of H_2 during growth of *Methanosarcina* spp. on acetate. Appl. Environ. Microbiol. 49:247-249.
10. **McInerney, M. J., and M. P. Bryant.** 1981. Anaerobic degradation of lactate by syntrophic associations of *Methanosarcina barkeri* and *Desulfovibrio* species and effect of H_2 on acetate degradation. Appl. Environ. Microbiol. 41:346-354.
11. **McInerney, M. J., M. P. Bryant, and N. Pfennig.** 1979. Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. Arch. Microbiol. 122:129-135.
12. **Phelps, T. J., R. Conrad, and J. G. Zeikus.** 1985. Sulfate-dependent interspecies H_2 transfer between *Methanosarcina barkeri* and *Desulfovibrio vulgaris* during coculture metabolism on acetate or methanol. Appl. Environ. Microbiol. 50:589-584.
13. **Powell, G. E.** 1983. Interpreting the gas kinetics of batch cultures. Biotechnol. Lett. 5:437-440.
14. **Smith, M. R., and R. A. Mah.** 1978. Growth and methanogenesis by *Methanosarcina* strain 227 on acetate and methanol. Appl. Environ. Microbiol. 36:870-879.
15. **Trüper, H. G., and H. G. Schlegel.** 1964. Sulfur metabolism in *Thiorhodaceae*. I. Quantitative measurements on growing cells of *Chromatium okenii*. Antonie van Leeuwenhoek J. Microbiol. Serol. 30:225-238.
16. **Weimer, P. J., and J. G. Zeikus.** 1977. Fermentation of cellulose and cellobiose by *Clostridium thermocellum* in the absence and presence of *Methanobacterium thermoautotrophicum*. Appl. Environ. Microbiol. 33:289-297.
17. **Zeikus, J. G.** 1977. The biology of methanogenic bacteria. Bacteriol. Rev. 41:514-541.
18. **Zeikus, J. G., P. J. Weimer, D. R. Nelson, and L. Daniels.** 1975. Bacterial methanogenesis: acetate as a methane precursor in pure culture. Arch. Microbiol. 104:129-134.

ENZYMATIC AND INITIAL GENETIC STUDIES ON METHANOGENS

Nancy W. Y. Ho
Laboratory of Renewable Resources Engineering
A. A. Potter Engineering Center
Purdue University
West Lafayette, IN 47907

ABSTRACT

Due to the extreme sensitivity to oxygen and unique physiology of the methanogens, it is exceedingly difficult to apply classical methodology to methanogens for their strain improvement. The recent advances in genetic engineering technology have made it possible to design new genetic approaches for the development of improved methanogens for methane generation. In order to apply this new technology to the methanogens, the construction of suitable cloning vectors for the transformation of the desired methanogens is essential. An effective cloning vector should contain at least two elements: a replication origin and a gene to serve as a selection marker. One of our efforts is to develop suitable selection markers for the establishment of transformation systems for the desired methanogens. Recently we have been able to demonstrate that the bacterial kanamycin resistance gene can be an excellent selection marker for the development of transformation systems for some of the methanogens. Efforts have been made towards the construction of effective kanamycin resistance gene based cloning vectors for genetic engineering of the methanogens.

ENZYMATIC AND INITIAL GENETIC STUDIES ON METHANOGENS

INTRODUCTION

The long term goal of this sub-program is to apply recombinant DNA technology to improve the capability of the methanogen bacteria to produce methane. Because of their extreme sensitivity to oxygen and unique physiology, the study of genetics of the methanogens by classical methodology has been extremely slow. For example, very few mutants have been isolated over the years. The recent advances in genetic engineering technology have made it possible to modify microorganisms by gene cloning to improve their capability. Particularly, this technology is equally applicable to those microorganisms such as the methanogens with little knowledge about their genetics. In general, genetic engineering technology can be applied to any organism as long as a DNA-mediated transformation system can be established for the organism. Therefore, the development of a transformation system for a methanogen is absolutely essential for the application of recombinant DNA techniques to improve its capability for more effective methane production. Hence, up to now, we have devoted our major efforts to the establishment of such systems for the methanogen species which are significant for anaerobic digestion of MSW and waste water.

There are two types of DNA-mediated transformation of living cells, the integrative transformation, and the plasmid-mediated, non-integrative transformation. The integrative transformation allows the cloned gene to be inserted into the chromosome; the plasmid-mediated, non-integrative transformation permits the gene to be cloned on the plasmid which can replicate autonomously and be stably maintained as an extrachromosomal element. The establishment of a plasmid-mediated, non-integrative transformation system for an organism is most ideal for the study of the genetics and biology of the organism at the molecular level. It also allows the determination of the enzymatic reaction which is the rate limiting step in a biosynthetic pathway, followed by the subsequent cloning of the specific gene on a high copy-number plasmid to overproduce the specific enzyme catalyzing the rate-limiting reaction and improve the production of the special product.

The establishment of a plasmid-mediated, non-integrative transformation system for an organism requires the construction of suitable cloning vectors that function as the carriers of the desired gene to be cloned. An effective cloning vector needs to contain at least two essential elements; a replication origin and a gene to serve as a selection marker.

A cryptic (endogenous) plasmid from a methanogen species will be the ideal replication origin for the construction of cloning vectors for that particular species, possibly also for other methanogens. So far, only three species of the methanogens are found to contain plasmid-like elements (Thomm et al. 1983; Wood et al. 1985; Meile et al. 1983), and the best characterized plasmid is the one isolated from *M. thermoautotrophicum* strain *Marburg* (Meile et al. 1983). Hence, *M. thermoautotrophicum* will be chosen as the model methanogen for the development of a transformation system.

A dominant selection marker such as an antibiotic resistance gene is an ideal selection marker for the construction of cloning vectors, particularly for those

microorganism like the methanogens which are difficult to isolate mutants. The availability of vector-borne, selectable genes that confer an antibiotic-resistance phenotype upon their host has been one of the primary reasons for the rapid and widespread use of recombinant DNA techniques in bacteria, particularly in *E. coli*. Antibiotic selection procedures are powerful because of their simplicity. The only requirement for selection is that cells that have picked up the vector-borne, antibiotic-resistance gene during transformation grow in the presence of antibiotic; all non-transformed cells should be killed or prevented from growing. One drawback is that methanogens are resistant to most antibiotics such as Ampicillin and tetracycline that are commonly used as selective agents for bacteria transformation. However, many eukaryotic species, such as yeasts and plants which are also resistant to most antibiotics are sensitive to antibiotic geneticin G418 (Webster and Dickson 1983; Colbere-Garapin et al. 1981). Furthermore, geneticin can be inactivated by the enzyme aminoglycoside phosphotransferase (APH) encoded by the bacterial kanamycin resistance gene (km^R). Geneticin and km^R have been used as a selection system to establish transformation systems for various yeasts (Webster and Dickson 1983; Ho et al. 1984). Hence, the use of Geneticin (G418) and km^R as the selection system for the establishment of a gene cloning system for the desired methanogens has been attempted.

MATERIALS AND METHODS

Strains

M. thermoautotrophicum strain *Marburg* (DSM 2133) was obtained from the German Collection of Microorganisms, Gottingen, F.R.G. *M. thermoautotrophicum* was cultivated according to Zeikus and Wolfe (1972), and *Ms. mazei* was cultivated according to Mah (1980). *Escherichia coli* k12 strain GM8 and *E. coli* MC 1116 (Casadaban et al. 1983) were used for transformation for the construction of recombinant DNA containing methanogen plasmid pME2001.

Plasmids

Plasmid pME2001 was isolated from *m. thermoautotrophicum* as described by Meile et al. (1983). Plasmids pUCKm6, pLX101, and pLX10-19 were all constructed in our laboratory.

Enzymes and General Methods

Restriction endonuclease, T4 DNA ligase, Klenow fragment of *E. coli* DNA polymerase I, and other enzymes used for gene splicing were obtained from Bethesda Research Laboratories (BRL) or New England Biolab. *E. coli* transformation, restriction endonuclease digestion, and other recombinant DNA manipulations were carried out as described previously (Rosenfeld et al. 1984,Stevis and Ho 1985).

Chemicals and Gases

Antibiotic G418 sulfate (geneticin) was purchased from Gibco Laboratories, Madison, WI. All other chemicals were of reagent grade. Gases were obtained from Matheson Scientific, Inc., Joliet, IL.

RESULTS AND DISCUSSION

The Effect of Geneticin on the Methanogens

The effect of antibiotic G418 on *M. thermoautotrophicum*, *Ms. mazei, and Methanococcus voltae* was studied by culturing these strains in liquid medium in the presence of various concentrations of G418 sulfate. The effect of antibiotic geneticin on the growth of *M. thermoautotrophicum* and *Ms. mazei* is shown in in Figures 1 and 2. Among these three strains of methanogens, *M. thermoautotrophicum* is most sensitive to the antibiotic and its growth can be totally inhibited by the presence of less than 50 μg/ml geneticin in the culture medium. *M. voltae* is least sensitive to the antibiotic; its growth will not be totally inhibited even in the presence of 300 μg/ml geneticin in the culture medium (for detail, see Ref. Feldman and Ho 1985).

The Effect of Aminoglycoside Phosphotransferase Treated Geneticin on the Methanogens

We have shown in Figures 1 and 2 that geneticin can inhibit the growth of *M. thermoautotrophicum*, which is the parent species for pME2001 and *Ms. mazei* which is an acetate-utilizing methanogen. In order for geneticin to be useful as a selective agent for the development of a transformation system for *M. thermoautotrophicum* and *Ms. mazei*, it has to prove that geneticin inactivated by the enzyme encoded by a plasmid-borne kanamycin resistance gene can no longer inhibit the growth of these methanogens. Figure 3 shows that geneticin treated with the enzyme aminoglycoside phosphotransferase produced by the km^R can no longer inhibit the growth of both *M. thermoautotrophicum* and *Ms. mazei*. The experiments have been repeated a number of times and the same results were obtained (these results have been reported in a methanogen meeting and a paper will be published on this subject, manuscript in preparation).

Construction of E. coli-Methanogen Shuttle Plasmids

Since we have demonstrated that antibiotic geneticin and the bacterial kanamycin resistance gene can be used as a selection system for the development of a plasmid-mediated transformation system for *M. thermoautotrophicum*, and *Ms. mazei*. We proceeded to construct *E. coli*-methanogen shuttle vectors which should contain both the bacterial kanamycin resistance gene and the methanogen plasmid pME2001 (Meile et al. 1983).

Our strategy to accomplish this is to insert the XhoI linearized methanogen plasmid into SalI linearized *E. coli* plasmid pUCKm6. As a result, we constructed plasmid pLM1 as shown in Figure 4. Although pLM1 contains an insert which is identical in size with pME2001, the insert does not have the same restriction sites with pME2001. Subsequently, the same experiments have been repeated a number of times by inserting pME2001 into different *E. coli* plasmid and transforming different hosts (some of them are $r^- m^+$ and some of them are recA mutants). pME2001 derivatives containing insertions or deletions were cloned but not the intact pME2001. Nevertheless, plasmids such as pLM1 which contains a segment of methanogen chromosomal DNA (demonstrated by autoradiography) should be very useful for carrying out integrative transformation of *M. thermoautotrophicum* (some of the results have been published in Ref. Feldman and Ho 1985). Nevertheless, recently we have attempted

once again to insert pME2001 (by ligation) into the *E. coli* plasmid pLX101. pLX101 is a direct selection plasmid that we constructed recently (Figure 5) (Stevis and Ho 1987). The resulting ligation products were used to transform MC1116, an *E. coli* recA host (possible also k^- m^+). After screening more than 30 positive transformants, we found two of them contained inserts with a size identical to pME2001. These plasmids are designated pLX101(ME2001)(3) and pLX101(ME2001)(7) (Figure 6). pLX101(ME2001)(3) has been characterized further and it apparently contains most restriction sites as reported for pME2001 (Figure 7). However, pLX101(ME2001)(3) seems still to contain some minor rearrangements. A detailed restriction map will be constructed for pLX101(ME2001)(3) and pME2001 to further determine whether pLX101(ME2001)(3) does contain most of pME2001. Such a restriction map is very useful for subsequent sequence analysis of the plasmid.

The Development of Novel Techniques for the Isolation of Methanogen Promoters

There are several reasons which prompted us to develop techniques as such. Firstly, we had reason to believe that the bacterial km^R might not be expressed in the methanogens unless it was modified such as by replacing the bacterial promoter with a methanogen promoter. Secondly, recent study of the methanogen genomic structure showed that most methanogen promoters are located within 100 base pairs from the initiation codon of a gene. This indicates that the promoters can be easily located on a cloned methanogen gene. Thirdly, such a technique will have a broad use for the isolation of strong promoters as well as for the determination of the promoter region from a group of cloned genes. Recently it has been demonstrated that methanogen genes are assembled into large operons and the promoter for the operon is difficult to locate. Fourthly, our preliminary results indicated that quite a few of the 5'-non-coding regions of the methanogen genes contain DNA sequences recognizable by the *E. coli* RNA polymerase as promoters

Initially our approach was to apply the *lacZ* fusion technique for the isolation of the promoters of the methanogens (Casadaban et al. 1983). We found that transformation of MC116 (a *lacZ* mutant) with HaeIII digested *M. theromautotrophicum* DNA ligated to SmaI digested pMC1403 (a plasmid containing a defective *lacZ* gene without its promoter and 5' terminal coding sequences (Casadaban et al. 1983)), and resulted in the generation of quite a few blue (Lac^+) colonies on the X-gal indicator plates (Casadaban et al. 1983). Several such methanogen partial gene promoter fragments have been characterized by restriction digestion and two of them have been sequenced. This indeed provides a very useful method for the isolation promoters from the methanogen genes.

The only disadvantage of the above described method is that the fusions may contain different lengths of the 5'-coding sequences of different genes, from a few to over 1,000 (Casadaban et al. 1983). This makes the location of the true initiation sites laborious and difficult. Currently we are experimenting with a similar technique by using pLX10-19. pLX10-19 is a derivatives of pLX101 (Figure 5) but it contains a defective *xyl*A gene. When *xyl*A in pLX10-19 is properly fused to a 5'-coding sequence and promoter, the resulting gene can synthesize xylose isomerase. The clones containing such a fused gene will be red on the MacConkey indicator plates, the rest of the clones containing the original pLX10-19 will be white on the indicator plates. The *xyl*A fusions have many advantages over the *lacZ* system. One of them might be that

only short 5'-coding sequences could be fused to the defective *xyl*A and result in the synthesis of functional xylose isomerase. This will make it easy to predict the region containing the methangen promoters.

The above are the highlights of our research related to anaerobic digestion of MSW and waste water. We have performed enormous additional research such as construction of gene banks for *M. theromautotrophicum* and *Ms. mazei*, the isolation of enzymes for antibody isolation and for the sequence determination. The sequence information will be used for the synthesis of the probes for the isolation of the desired genes, etc.

In conclusion, we like to emphasize the special usefulness of genetic engineering technology towards the strain improvement of the methanogens. After the initial stage of the development of the necessary techniques, new methanogen strains with improved methane-productivity should be able to be constructed.

In the near future, the following research will be continued: transformation of both *M. theromautotrophicum* and *Ms. mazei* with various plasmids containing the km^R; development of new techniques for the isolation of methanogen genes containing the strong promoters to be used for the improvement of methanogen genes for improved methane production; construction of detailed restriction map for the methanogen plasmids pME2001; and sequence analysis of the latter plasmid, etc.

REFERENCES

Casadaban, J. J. et al. 1983. *Methods Enzymol* 100:293-308.

Colbere-Garapin, F. et al. 1981. "A New Dominant Hybrid Selection Marker for High Eukaryotic cells," *J. Mol. Biol.* 150:1-14.

Feldman, D., and Ho, N.W.Y. 1985. "Antibiotic Geneticin (G418) as the Selection Agent for Methanogen Transformation," *Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals*, Argonne National Laboratory, A. A. Antonopoulos, ed. 187.

Feldman, D., and Ho, N.W.Y. 1985. "Construction of Plasmids for the Transformation of *M. theromautotrophicum*," *Biotechnol. Bioeng. Symp.* 15:235-240.

Ho, N.W.Y. et al. 1984. "Development of a Cloning System for *Candida* Species," *Biotech. Bioeng.* 14:295-300.

Mah, R. A. 1980. "Isolation and Characterization of *Ms. mazei*," *Current Microbiology* 3:321-326.

Meile, L. et al. 1983. "A Plasmid in *M. theromautotrophicum*," *Mol. Gen. Genet.* 191:480-484.

Rosenfeld, S. A., Stevis, P. E., and Ho, N.W.Y. 1984. "Cloning and Characterization of the xyl Genes from *E. coli*," *Mol. Gen. Genet.* 194:410-415.

Stevis, P. E., and Ho. N.W.Y. 1985. "Overproduction of xylose Isomerase in *E. coli* by Cloning the D-Xylose Isomerase Gene," *Enzyme Microb. Technol.* 7:592-596.

Stevis, P. E., and Ho, N.W.Y. 1987. "Positive Selection Vectors Based on Xylose Utilization Suppression," *Gene* 55:67-74.

Thomm, M., Altenbuchner, and Stetter, K. O. 1983. "Evidence for a Plasmid in a Methanogenic Bacterium," *J. Bacteriol.* 153:1060-1063.

Webster, T. D., and Dickson, R. C. 1983. *Gene* 26:243-252.

Wood, A. G. et al. 1985. "A Newly-Isolated Marine Methanogen Harbors a Small Cryptic Plasmid," *Arch. Microbiol.* 142:259-261.

Zeikus, J. G., and Wolfe, R. S. 1972. *J. Bacteriol.* 109:707-713.

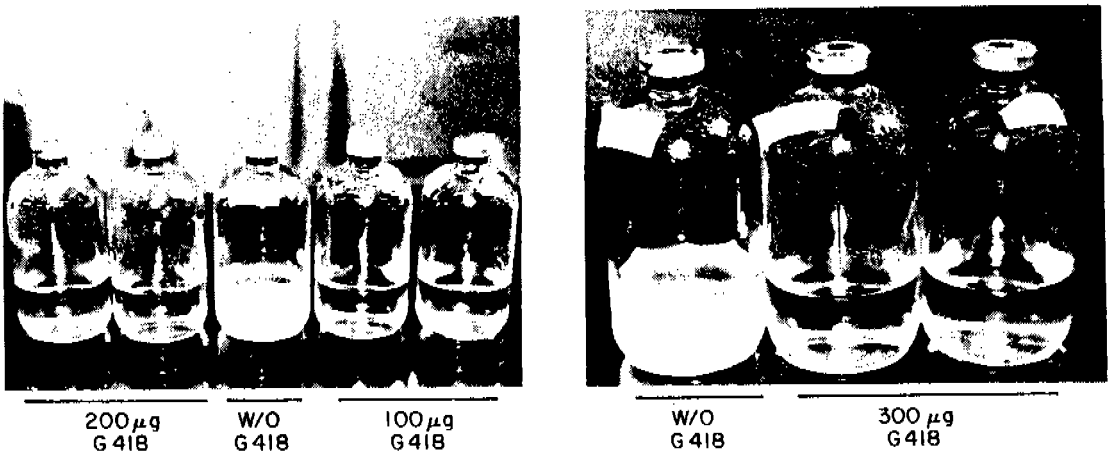


Figure 1. The effect of antibiotic geneticin on *M. thermoautotrophicum.*

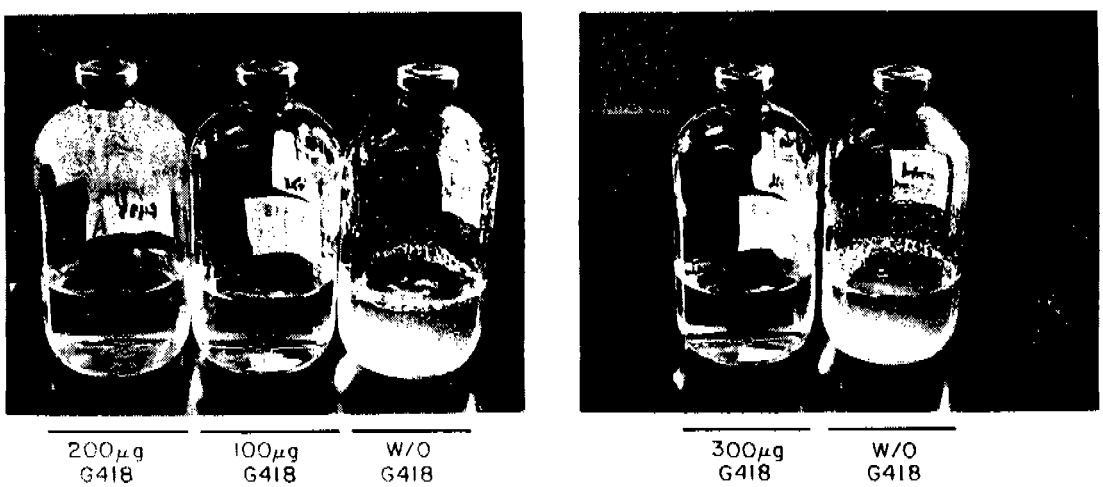


Figure 2. The effect of antibiotic geneticin on *Ms. mazei.*

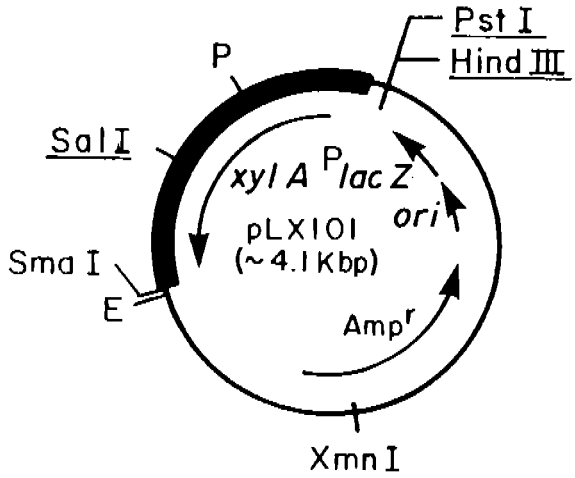


Figure 5.

3 7

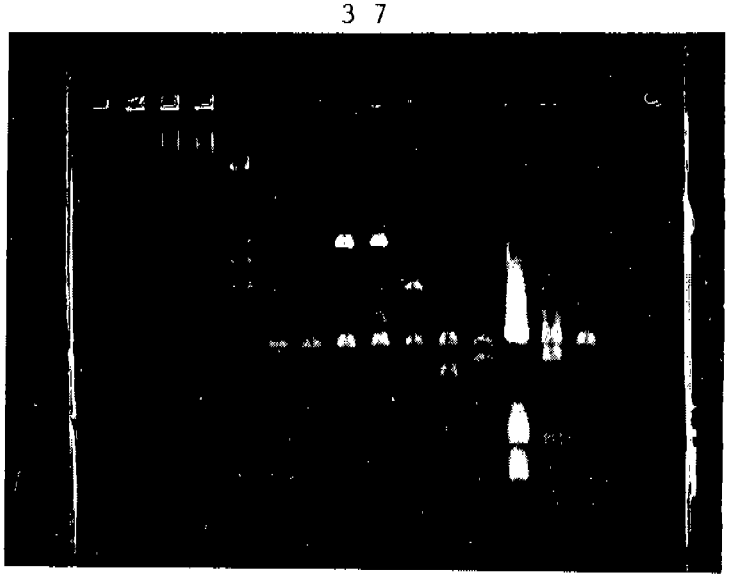


Figure 6. Restriction analysis of recombinant plasmids for containing pME2001. Numbers 3 and 7 contain inserts identical in sizes with pME2001.

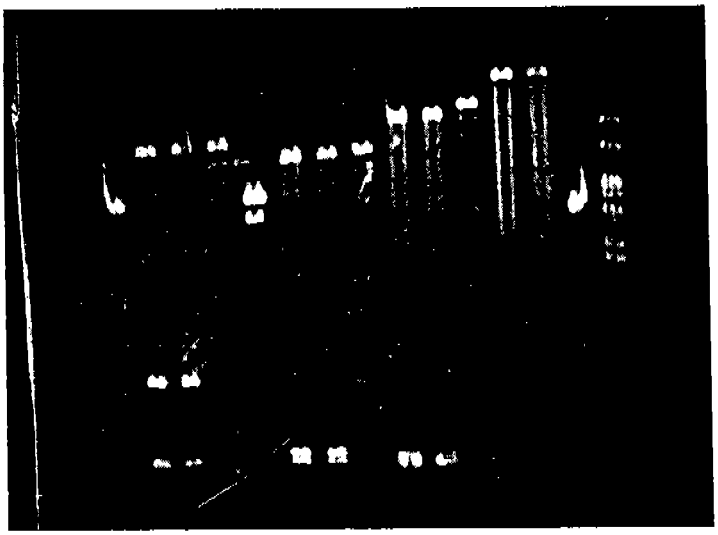


Figure 7. Restriction analysis of pLX101(ME2001) numbers 3, 7, and 11. Numbers 3 and 7 contain inserts with identical AvaI, HindIII, PvuII, BalI HpaI sites with the intact pME2001.

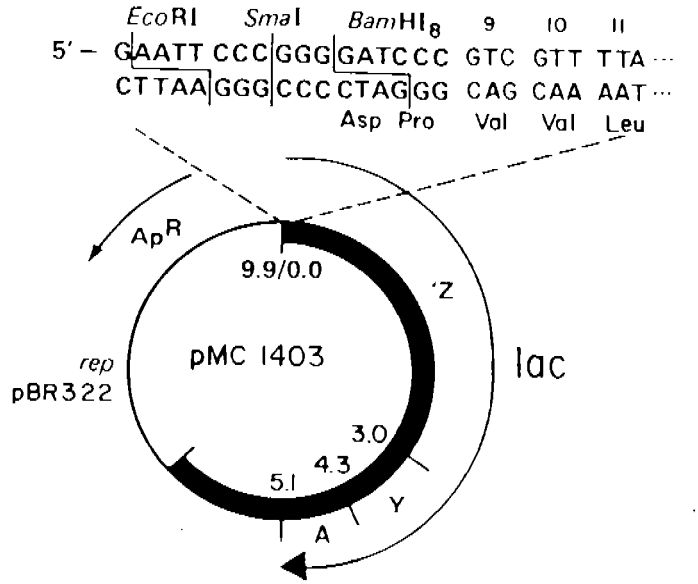


Figure 8. Construction of methanogen DNA-*lacZ* fusions.

EFFECTS OF TRACE METALS ON METHANOGENESIS AND CONTROL OF SULFATE REDUCING BACTERIA

Ross E. McKinney
N.T. Veatch Professor of Environmental Engineering
Department of Civil Engineering
University of Kansas
Lawrence, Kansas 66045

ABSTRACT

The research over the past three years has demonstrated the need for various trace metals in anaerobic metabolism of basic organics. Sodium butyrate was used as the first organic to be studied because it required a complex microbial population for metabolism. Systems were initially studied at 37C and 55C with 20-25C temperature systems examined last. Trace metal requirements were used at all temperature levels. Sulfate reduction was examined with sodium butryrate first and then with sodium acetate. While it had been indicated that molybdate inhibited sulfate reducation without affecting methane generation in coastal muds, the data on controlled digesters showed that molybdate was toxic to both the sulfate reducing bacteria and the methane bacteria. It was found that survival depended upon the ability to obtain energy from the organic substrate.

Energy-synthesis relationships were examined for sodium butyrate under different HRT-SRT conditions in an effort to understand metabolic relationships. It was found that all systems showed the same solids accumulation each day with 36C digesters showing less solids than the 24C digesters. The lower temperature affected the rates of metabolism and the rates of endogenous respiration. Retention of the microbes is critical to high rate metabolism. It was found that the anaerobic bacteria flocculated the same as aerobic bacteria, permitting microbial retention of dispersed systems. Initial fixed media studies indicated bacteria retention was also by flocculation and sedimentation in the media with little adherence of the bacteria to the media.

EFFECTS OF TRACE METALS ON METHANOGENESIS AND CONTROL OF SULFATE REDUCING BACTERIA

INTRODUCTION

The research of Schonheit, Moll and Thauer in 1979 showed that nickel, cobalt and molybdenum were essential trace elements for thermophilic methane bacteria. While iron had long been known as an essential element, it was the nickel that created the stir. Nickel was a toxic heavy metal that had never been shown to be of value in bacterial metabolism. It was not long before Murray and Van den Berg in 1981 showed that nickel, cobalt and molybdenum showed a positive effect on fixed film, methanogenic reactors. Speece, Parkin and Gallagher confirmed that nickel stimulated anaerobic digestion. Qualitative data are valuable; but quantitative data are essential if full scale systems are to be designed and applied in wastewater treatment.

Trace Metals

This study began three years ago using sodium butyrate as the organic substrate to provide a complex microbial population. Sodium butyrate is a simple, soluble fatty acid salt that must be metabolized initially by beta oxidation to acetic acid before being broken to methane and carbon dioxide. The initial metabolism requires that carbon dioxide be reduced to form methane and water. A second set of bacteria metabolize the acetic acid to methane and carbon dioxide. Two different groups of methane bacteria are involved in this metabolism.

The initial seed sludge was Topeka, Kansas, digesting sludge. A series of five, 1.5 L, batch-fed digesters were started with sodium butyrate at 1.73 g/d COD. Efforts to develop good operations with a 6 day hydraulic retention time (HRT) and solids retention time (SRT) at 37C were not successful. Gas production dropped and the SRT was increased to 15 days to retain the microbes. Iron, nickel, zinc and manganese at 1.0 mg/L were added with mixed results. Iron gave some increase in metabolism with zinc showing an increase in the iron unit and a decrease in the nickel and cobalt digesters. Manganese gave a slow increase in metabolism in all digesters. The most significant increase in gas production came when sodium sulfide was added to the digesters along with 1.0 mg/L copper. It had been assumed that sulfates were being reduced to provide the correct environment for the bacteria. The gas showed 83% methane; but the volatile acids increased steadily, indicating incomplete metabolism. After the addition of sodium sulfide on a daily basis, the volatile acids dropped from 7800 mg/L to 260 mg/L in two weeks in one of the digesters. Examination of the solubility products of the heavy metal sulfides indicated that they ranged from 10^-15 for manganous sulfide to 10^-47 for cuprous sulfide. With over 60 mg/L sulfides in the digesters, the trace metals were essentially removed from solution. The positive response to the trace metals and sulfides prompted a reduction in SRT down to 6 days. Gas production fell off, indicating a loss of active microbes. The SRT was allowed to increase while the HRT was slowly reduced to 3 days. The

systems all came to good operations with essentially 99% metabolism of the butyrate fed on a daily basis. It appeared that iron, nickel, cobalt, molybdenum, manganese, copper and zinc were needed to produce maximum metabolism of the butyrate.

A second series of digesters were operated at 55C to determine how thermophilic bacteria responded to the trace metals. The Topeka, Kansas, digesting sludge was used as the source of microbes with good metabolism of the initial feeding of sodium butyrate in just four days. Daily feeding quickly established a good microbial population in a few days. The thermophilic bacteria were more sensitive to changes than the mesophilic bacteria, necessitating reseeding twice during this phase of the study. It was necessary to operate at 15 days HRT and as long an SRT as possible to buildup the initial microbial population. It was established that the thermophilic bacteria also required the same trace metals as the mesophilic bacteria. Efforts to lower the HRT in the digesters showed that the daily loss of microbes limited the operational HRT. While the long SRT gave good bacterial flocculation and separation, the active bacteria tended to remain dispersed and were lost with the effluent removed each day.

Rapid Screening Technique. While the laboratory digesters were operating, a rapid screening technique was developed to help determine the impact of the different trace metals on metabolism. The rapid screening technique was essentially an anaerobic fermentation tube test using a 50 ml culture tube with an inverted vial to collect the gas produced. It was possible to use a single organic substrate in a series of tubes and to vary the trace metals in each tube both as to combinations and concentrations. The tubes were prepared in sets of 20 tubes and were inoculated with 1 ml of seed microbes from the effluents of the operating digesters. The tubes were sealed with a rubber stopper, taking care not to trap an air bubble in the tube. The tubes were incubated for one to two weeks at the desired temperature with gas production noted at regular intervals. At the end of each run, efforts were made to analyze the gas composition and to measure the pH. Eventually, the inverted vial was replaced with tubes having a septum cap so that gas samples could be taken more easily and the volume of gas could be better estimated.

Examination of sodium acetate metabolism indicated that a mixture of trace metals gave better gas production than the separate trace metals. The gas analysis showed essentially methane and nitrogen with only a trace of carbon dioxide. The nitrogen gas was primarily stripped from the water at the higher temperature used for incubation. The carbon dioxide was tied up as sodium bicarbonate formed by the degradation of sodium acetate. The pH of the units were around 8.4. With sodium butyrate as the substrate metabolism was strongly acidic when nickel was present. The pH dropped to 5.5 when nickel was the only trace metal. Chromium also depressed the pH. On the other hand iron showed a pH of 7.8. The nickel and the chromium stimulated the dehydrogenases and resulted in rapid accumulation of hydrogen since carbon dioxide was limited for reaction with the hydrogen.

Solids Retention Time. In an effort to determine the trace metal concentrations required in the feed, the five batch-fed digesters were operated under different environmental conditions. Digester 1 was set at a 3 day HRT and a 15 day SRT. Digester 2 was set at a 15 day HRT and a 30 day SRT. Digester 3 was set at 15 days HRT and 15 days SRT with Digester 4

set at 10 days HRT and SRT and Digester 5 set at 7.5 days HRT and SRT. The trace metals were set at 0.01 mg/L in the daily feed of sodium butyrate at 1.73 g COD. In two weeks the gas production was 720 ml/d in Digester 1, 770 ml/d in Digester 2, 740 ml/d in Digester 3, 600 ml/d in Digester 4 and 440 ml/d in Digester 5. As the SRT was reduced, the microbial populations were unable to sustain good metabolism. Trace metals were increased to 0.1 mg/L for the next two weeks. The gas production was 720 ml/d in Digester 1, 760 ml/d in Digester 2, 760 ml/d in Digester 3, 640 ml/d in Digester 4 and 160 ml/d in Digester 5. The trace metals were increased to 1.0 mg/L. The gas production was 560 ml/d in Digester 1, 770 ml/d in Digester 2, 620 ml/d in Digester 3 and 660 ml/d in Digester 4. Digester 5 failed and had to be restarted. It was apparent that the SRT was critical for good operations; but it was not apparent as to the trace metal concentrations. In an effort to delineate the desired trace metal concentrations, a series of continuously fed digesters were started.

The continuously fed digesters were started as batch fed digesters operating with a 15 day HRT and an SRT determined by loss of solids in the effluent. All five digesters showed about 700 ml/d gas volume at 1.73 g/d COD as sodium butyrate. The feed was increased to 3.46 g/d COD with Digester 1 receiving 0.0 mg/L trace metals, Digester 2 - 0.001 mg/L TM, Digester 3 - 0.01 mg/L TM, Digester 4 - 0.1 mg/L TM and Digester 5 - 1.0 mg/L TM. When the continuous feeding was started, the digesters averaged 1425 ml/d gas production. Gas production was measured with wet test gas meters. Efforts to control the feed pumps at 100 ml/d was quite difficult and the systems were shifted to 800 ml/d for better operations. The COD feed was increased to 6.92 g/d COD and then to 13.8 g/d COD, 17.3 g/d COD, 20.8 g/d COD and finally to 24.2 g/d COD. The daily gas production at 24.2 g/d COD was 1.74 L/d in Digester 1, 4.71 L/d in Digester 2, 7.3 L/d in Digester 3, 8.19 L/d in Digester 4 and 9.33 L/d in Digester 5. The COD load was increased to 27.7 g/d in Digesters 3, 4 and 5. Digester 3 failed and the COD load was raised to 31.1 g/d. After one week the gas production was 9610 ml/d in Digester 4 and 8810 ml/d in Digester 5. It appeared that 17.5 g/d COD was converted to methane in Digester 4 and 16.4 g/d in Digester 5. It was apparent that both digesters were loaded beyond their capacity and that there was little to gain from longer operations at these high organic loading rates. The 0.1 mg/L trace metal digester appeared to give slightly better results than the 1.0 mg/L TM digester.

The thermophilic digesters could not be operated on a continuously fed basis and had to be examined on a batch-fed basis. The systems could only be operated at a maximum of 3.46 g/d COD because of the limitations of the gas measurement bottles. There were not enough wet test gas meters for both studies. It was found that best operations occurred with a 5 day HRT and maximum solids retention. The 0.1 mg/L trace metal digester and the 1.0 mg/L TM digester gave the best results, confirming the mesophilic data.

Temperature Variations

The thermophilic digesters provided an opportunity to examine the effect of temperature shifts on the microbial populations and the resulting metabolism. The 0.1 mg/L TM digester and the 1.0 mg/L TM digester were found to give good operations with a 5 day HRT at 55C. The final day of operations at 55C produced 1475 ml gas in both digesters. The suspended

solids data showed 810 mg/L TSS with 59% VSS in the 0.1 mg/L TM digester and 2220 mg/L TSS with 60% VSS in the 1.0 mg/L TM digester. Even though the VSS were 2.8 times greater in the 1.0 mg/L TM digester, the rate of gas production indicated that the active mass was only 1.5 times greater. The methane in the gas was 88% for 95% COD conversion to methane. The data indicated rapid synthesis and endogenous respiration in the thermophilic digesters.

The two thermophilic digesters were moved from 55C to 37C with the same organic load, 3.46 g/d COD as sodium butyrate. The first day at 37C the 0.1 mg/L TM digester showed 425 ml of gas, only 29% of the previous days gas production. The 1.0 mg/L TM digester produced 675 ml of gas, 46% of the previous days gas production. The anticipated drop in gas production was to 29% of the 55C gas production, based on normal rate changes. Repeat feeding indicated incomplete metabolism with a buildup of unmetabolized volatile acids. The digesters were not fed for several days after the first week of daily feeding to allow the bacteria population to metabolize the accumulated volatile acids. The digesters acclimated to the new conditions over a 40 day period. The daily gas production reached 1275 ml/d in the 0.1 mg/L TM digester and 1350 ml/d in the 1.0 mg/L TM digester. With 85% methane in the gas the 0.1 mg/L TM digester had 2.62 g/d COD converted to methane; while the 1.0 mg/L TM digester had 2.81 g/d COD converted to methane. The volatile acids removed in the effluent accounted for 0.67 g/d COD in the 0.1 mg/L TM digester and 0.78 g/d COD in the 1.0 mg/L TM digester. The higher rate of gas formation in the 1.0 mg/L TM digester was related to acclimation to unmetabolised volatile acids that had built up in the digester. Although the digesters had not completely acclimated to 37C, they were moved to ambient temperature, 20C, to see how well they would respond. The gas production the first day was 210 ml in the 0.1 mg/L TM digester and 275 ml in the 1.0 mg/L TM digester. The initial response to the lower temperature was less than expected, indicating that there was a shock to the microbes. The gas production stayed quite low except for a few days when the air conditioning was turned off and the ambient temperature rose to 33C with good gas production. The digesters did not respond to longer periods between feeding. The feed COD was reduced to 1.73 g/d to reduce the accumulation of volatile acids. The HRT was increased to 15 days. After four months the 0.1 mg/L TM digester produced only 350 ml/d gas while the 1.0 mg/L TM digester showed 850 ml/d gas production.

The two digesters were shifted back to 55C from 22C. The gas production in the 0.1 mg/L TM digester rose to 825 ml with 1850 ml in the 1.0 mg/L TM digester. It was apparent that the thermophilic bacteria still maintained their ability to metabolize at 55C. Within a month both digesters were operating with 700 ml/d gas production. The two digesters showed little change over the next 5 months. Yet, complete metabolism of the volatile acids did not occur. The trace metals in the 1.0 mg/L TM digester were increased to 2.0 mg/L for a week and then to 3.0 mg/L for another week. The trace metals were increased to 11 mg/L with no significant change in the remaining volatile acids. The SRT was reduced to 15 days, the same as the HRT to allow the microbes to respond to increased wasting. The TSS was 1540 mg/L, 61% VSS, in the 0.1 mg/L TM digester and 3850 mg/L, 48% VSS, in the 1.0 mg/L TM digester. The 0.1 mg/L TM digester operated for almost four months before feeding was stopped. The digester had slowly deteriorated with a decreasing rate of gas production and

increasing volatile acids. The 1.0 mg/L TM digester actually was maintained at 11 mg/L TM for a month to see how the microbes responded to two turnover times, 86% displacement. The results were not positive so that the system was returned to 1.0 mg/L TM. The volatile acids showed an immediate rise. The digester was not fed to allow the volatile acids to be metabolized and the methane bacteria to accumulate for a few days. Since the total alkalinity was at 5880 mg/L with a pH 8.4, butyric acid was fed instead of sodium butyrate to allow the pH and alkalinity to drop. The volatile acids showed a definite drop and gas production increased rapidly. When the pH reached 7.2 with 2200 mg/L total alkalinity, the feed was shifted back to sodium butyrate. The digester showed a slow decrease in volatile acids for a month and then gave a slight rise. After 87 days the digester had complete displacement of the microbial solids. The TSS were measured over a 9 day period at 386 mg/L with 75% VSS. With 85% methane in the gas and 690 ml/d gas production during this period, the COD of the methane produced was 1.48 g/d. The effluent averaged 480 mg/L volatile acids, 0.05 g/d COD; and the effluent VSS gave another 0.04 g/d COD for a total of 1.57 g/d accounted for, 91%. Measured effluent COD data gave 0.07 g/d COD related to VSS and volatile acids instead of 0.09 g/d.

A rate study was made over two days to determine metabolism of the sodium butyrate. The second day showed 1.57 g/d COD converted to methane, raising the total COD accounted for to 1.64 g/d. The system was allowed to continue to metabolize after the second feeding. The gas production rose to a maximum of 1015 ml on the third day after feeding and then dropped back to 965 ml on the eighth day when TSS were measured at 408 mg/L with 283 mg/L VSS. It appeared that CO2 was being absorbed from the gas, causing the decrease in volume. The gas volume was down to 905 ml when the next TSS measurement was made on the 11th day. The TSS had dropped to 356 mg/L with 234 mg/L VSS, confirming that some endogenous respiration was indeed occurring. By the 13th day the gas volume had dropped to 875 ml. The digester was refed to see how active the remaining bacteria were. The response was negative 2 days with a small response the 3rd day. The maximum gas production was 615 ml on the 7th day after feeding. The gas volume dropped slowly over the next four days. When the digester was analyzed for the last time, the TSS were 316 mg/L with 188 mg/L VSS. The digester appeared to have a rapid rate of endogenous respiration with loss of the active bacteria, making it difficult to recover when it was not fed each day. The gas production curve showed that the bacteria metabolized the organics quite quickly and then underwent slow endogenous respiration with loss of activity. The high rate of endogenous respiration at 55C results in a minimum of excess microbial solids to be processed; but it requires careful control to maintain a viable population in the thermophilic range. Figure 1 is a plot of the gas production data during this critical part of the study and it shows the rapid synthesis metabolism as well as the slow rate of endogenous respiration.

Sulfate Reduction

Sulfate reduction has long been considered an important part of anaerobic digestion. Some sulfate reduction is necessary to provide the reduced environment for good methane fermentation. Yet, too much sulfides appear to be toxic to the methane bacteria. The equilibrium between sulfides in solution and in the gas has been shown to be pH related with sulfides being stripped with methane and carbon dioxide at low pH values

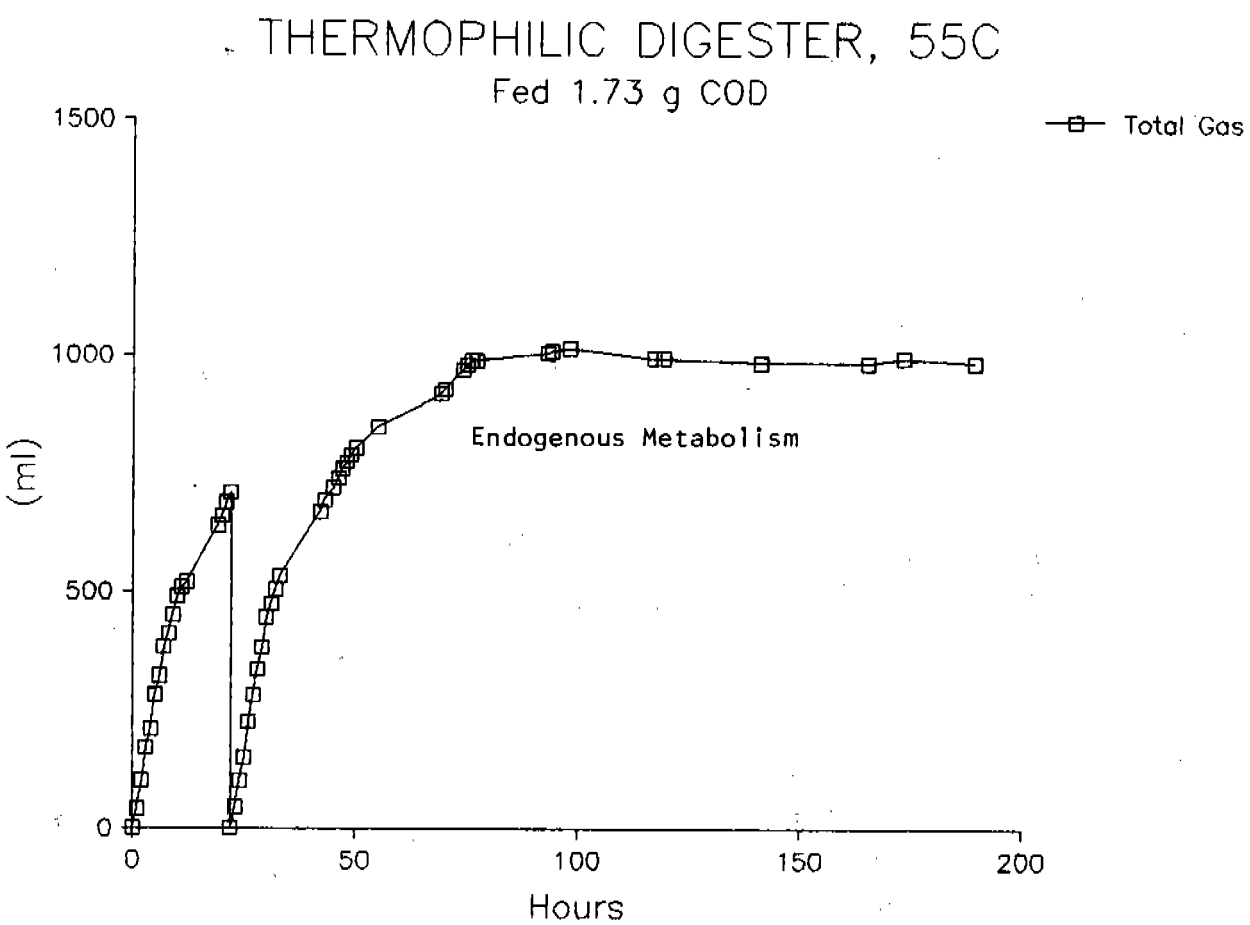


Figure 1 Gas Production at End of Study Showing Endogenous Metabolism

and retained as the bisulfide salts at high pH values. In 1958 Wilson and Bandurski found that molybdate blocked sulfate reduction. Taylor and Oremland in 1979 found that chromates were most effective in blocking the sulfate reduction reaction.

A series of five digesters were set up at 37C on sodium butyrate acclimated sludge with a 5 day HRT and a longer SRT as only the solids in the effluent were lost from the digesters. Digester 1 was the control digester and was only fed sodium butyrate normally. The Control digester operated normally, giving 790 ml/d gas with 86% methane, 1.65 g/d COD converted to methane. The Sulfate digester was fed 150 mg/d sulfates in addition to the butyrate and produced 720 ml/d gas with 89% methane, 1.55 g/d COD conversion to methane. The 150 mg/d sulfates allowed 100 mg/d COD to be oxidized rather than converted to methane and also tied up some of the carbon dioxide as sodium bicarbonate, giving less CO2 in the gas. The Iron digester was operated with the daily addition of 50 mg/d Fe to precipitate the sulfides as ferrous sulfide. The Iron digester averaged 750 ml/d gas with 87% methane, 1.58 g/d COD conversion to methane. It appeared that the Iron digester had 70% sulfate reduction. The Molybdenum digester was fed at 10 mg/D Mo with gas production dropping below that of the Control. Efforts to add molybdenum on a daily basis failed as it was toxic to the methane bacteria. Sulfide analyses showed 140 mg/L sulfides accumulating in the digester. The Chromium digester was operated at 80 mg/d at first and then dropped to 20 mg/d when the 80 mg/d proved toxic. The 20 mg/d chromium was not toxic even to the sulfate reducing bacteria which continued to reduce the sulfates.

Acetate Fed Digesters. The next phase of the sulfate reduction study was concerned with setting up five digesters metabolizing various combinations of sodium acetate and acetic acid. After acclimation the digesters were operated at 37C with a 5 day HRT and 3.2 g/d COD. By having 20%, 40%, 60%, 80% and 100% sodium acetate in the five different feeds, the pH of the digesters were 7.5, 7.7, 7.9, 8.2 and 8.6 at the end of each daily cycle. Batch feeding resulted in acid conditions that forced out CO2 in large volumes until the acetate was metabolized and the carbon dioxide was reabsorbed from the gas bottle to form sodium bicarbonate. The digesters were mixed by magnetic stirrers every other hour for an hour for 20 hours and then settled for four hours prior to drawing off the effluents. The digesters were fed 60 mg/d sulfates and then 120 mg/d sulfates to allow the sulfate reducing bacteria to grow up in competition with the methane bacteria. The pH 8.5 digester showed 46 mg/d sulfates in the effluent, 62% reduction. The pH 8.1 digester had only 5.4 mg/d sulfates remaining, 95% reduction. The pH 7.8 digester contained 11 mg/d sulfates in the effluent, 91% reduction. The pH 7.5 digester had 40 mg/d sulfates, only 66% reduction. The pH 7.2 digester had 27 mg/d sulfates, 78% reduction.

The sulfates fed were increased to 240 mg/d and then to 480 mg/d and to 960 mg/d and finally to 1920 mg/d. The pH 8.4 digester gave 1370 ml/d gas with 90% methane, 93% COD conversion, and 1770 mg/d sulfates in the effluent for only 8% reduction. The pH 8.1 digester gave 1430 ml/d gas with 86% methane, 93% COD conversion, and 1560 mg/d sulfates in the effluent for 19% reduction. The pH 7.7 digester produced 1550 ml/d gas with 81% methane, 95% COD conversion, and showed 1390 mg/d sulfates in the effluent, 28% reduction. The pH 7.6 digester had 1800 ml/d gas with 82%

methane and 1620 mg/d sulfates, 16% reduction. The pH 7.3 digester gave 1820 ml/d gas with 78% methane and the effluent contained 1440 mg/d sulfates, 25% reduction. The data with acetate showed that the methane bacteria were able to compete with the sulfate reducing bacteria under the batch feeding conditions. Sodium lactate was substituted for sodium acetate since lactate had been noted as a better substrate for sulfate reducing bacteria. The sulfate reduction ranged from 54% at the highest pH level to a maximum of 88%. There is no doubt that changing the substrate gave the sulfate reducing bacteria an advantage over the methane bacteria.

Fixed Media. With fixed media digesters used to retain the bacteria, a study was made to determine if sulfate reducing bacteria could adversely affect the digestion process once an active microbial population of methane bacteria had accumulated in the fixed media digesters. Five different types of fixed media were used in five different digesters: polyethylene, iron, glass, polypropylene and mixed bed ion exchange resins. Four of the digesters had 0.5 L mixing volume under 1.0 L fixed media. Mixing was supplied by magnetic stirring bars operated one hour on and one hour off for 20 hours and then having four hours settling. The mixed bed ion exchange resin was too small to be retained above the mixing zone, requiring hand mixing about once an hour for the normal 8 hour day and then quiescent settling the rest of the time.

The digesters were started with sodium butyrate at 3.4 g/d COD with a 6 day HRT and a longer SRT, depending upon daily solids losses. After the system was acclimated to sodium butyrate, peptone was added as a soluble protein to raise the COD to 6.3 g/d. Next, glucose was added to raise the COD to 8.4 g/d. Soluble starch was substituted for glucose, raising the COD to 8.7 g/d. There was essentially complete metabolism in all digesters with an average daily gas production at the end of this phase of 4120 ml/d at 75% methane. The total alkalinity at 0.0 mg/L volatile acids was 7060 mg/L. The sodium sulfate concentration was raised to 2000 mg/L and then the daily feed added 500 mg/d to keep the sulfates at 2000 mg/L if they were not reduced. It was estimated that the gas reduction with complete sulfate reduction would be about 740 ml/d at 37C with an increase of alkalinity of 2820 mg/L. After four weeks operation the average gas production was 3940 ml/d, a decrease of 180 ml/d, indicating 24% reduction. The alkalinity increased to 8800 mg/L, 1740 mg/L increase, indicating 62% reduction.

While there was some sulfate reduction, it was not complete. The soluble starch was removed and lactic acid was substituted, giving 8.5 g/d COD in the feed. The sulfate addition remained the same. The gas production after 35 days was higher than expected. It was noted that considerable CO2 was given off after feeding acid. The large volume of gas required emptying the gas displacement bottle twice with loss of the excess CO2. Gas analyses were always made on the last gas volume produced, giving a higher than normal methane fraction. It was not possible to use the gas production data as expected for evaluation. In an effort to stimulate the sulfate reducing bacteria, the organics were replaced completely with lactic acid. The metabolism of sodium sulfate produced all the alkalinity in the digesters. The addition of 3.0 g/d sodium sulfate gave a drop in gas production from 5820 ml/d to 5700 ml/d while the alkalinity rose from 7910 mg/L to 9090 mg/L. Sulfate data indicated over 92% sulfate reduction. The sulfate fed was increased to 4.5 g/d and then to 6.0 g/d. The mixed

resin digester failed at 6.0 g/d sodium sulfate; but the four mixed units showed an increase in alkalinity to 19,700 mg/L and a decrease in gas production to 4390 ml/d. The sulfate reduction was 95% complete. Examination of the gas about one hour after feeding showed 68% CO_2, 11% CH_4 and 18% H_2S. The stripping of the hydrogen sulfide with the gas and its removal from the system allowed the units to operate with a total sulfide concentration of 270 mg/L in the liquid phase at a pH of 8.3 at the end of the 24 hour feed period. The data definitely indicated that with microbial accumulation in fixed media digesters, almost complete sulfate reduction would be obtained.

Energy, Synthesis and Endogenous Metabolism

The key to anaerobic systems lies in the controlled metabolism of the mixed microbial population in the digesters. The bacteria metabolize the organics to obtain energy for synthesis. Because anaerobic reactions yield very little energy for the bacteria, synthesis of new cell mass is small compared to aerobic metabolism. Increasing the cell mass can only be obtained by retaining the bacteria in the system; but endogenous respiration and the hydraulic flow rate both act to reduce the active microbial mass available to respond to the new organic feed. Very little data have been obtained on bacterial synthesis under anaerobic conditions over a sufficiently long period of time to provide valid measurements. Even less data are available on the rates of endogenous respiration and the effect of temperature on the rate of endogenous respiration.

A study was made with sodium butyrate as the feed with varying HRT-SRT periods to provide a range of data. The mesophilic digesters were kept at 36C and had 10 days HRT-SRT, 12.5 days HRT-SRT, 15 days HRT-SRT, 20 days HRT-SRT, 30 days HRT-SRT and 45 days HRT-SRT. The daily feed COD was 3.46 g/d. After 88 days operation, a rate study was made with gas data recorded hourly and suspended solids data collected at the start and the finish of the rate study. All of the digesters gave good gas production, averaging 1800 ml/d gas with 85% methane. The initial rates of gas production indicated that the 20 day HRT-SRT digester had the most active bacterial mass with the 30 day HRT-SRT second, the 45 day HRT-SRT third, the 15 day HRT-SRT fourth, the 10 day HRT-SRT fifth and the 12.5 day HRT-SRT sixth. The retention pattern was not as clear as hoped for. The VSS data indicated that there was no significant difference in the daily VSS increase except with the 12.5 day HRT-SRT digester. The VSS increased over the other five digesters was 52 mg/d. Regardless of the SRT, the VSS averaged 26 percent of the maximum expected synthesized VSS. Since metabolism was complete in these units, 3.46 g/d COD was metabolized with 98% conversion to methane. A parallel study at ambient temperature, 24C, indicated greater VSS accumulations for the same COD feed and SRT. The VSS increase averaged 69 mg/d at the lower temperature, 34 percent of the maximum theoretical VSS expected. The initial rate of gas production at 36C averaged 105 ml/hr; while the 24C gas production rate was 75 ml/hr. Since the amount of cell mass produced per unit of substrate metabolized was constant, the differences in rates of metabolism and the amount of endogenous respiration produced the different solids quantities measured. The rates of reactions are very important in determining optimum loading conditions for anaerobic systems and the mass of active bacteria maintained in the digesters sets the maximum rate of metabolism.

Further Studies

The proper design and operation of anaerobic digesters lies in understanding the basic microbiology and biochemistry the same as it has with aerobic wastewater treatment systems. Much basic research remains to establish quantitative energy synthesis relationships for the major types of organics found in waste systems. The rates of endogenous respiration and the quantities of endogenous mass accumulating need to be evaluated and established so that full size system operations can be predicted with greater precision than at the present time. Initial examination of the biochemistry of some simple organics has shown that basic energetics determine the extent of metabolism each group of bacteria are able to carry out and the amount of cell mass that will be produced. Complex organic wastes require a far more complex set of bacteria than simple organic wastes.

Maintenance of active microbes in the systems has been shown to be essential for complete metabolism of the organics. Essentially complete metabolism of organic substrates has been obtained when the microbial mass is adequate. Loss of microbes in the effluent or by extended endogenous respiration resulted in incomplete metabolism. These studies indicated that anaerobic bacteria form floc the same as aerobic bacteria and can be kept in the system by sedimentation. Preliminary studies with fixed media systems confirmed that the bacteria are retained by flocculation and sedimentation with few attached bacteria. Additional studies need to be made on microbial attachment to surfaces and the impact of organic loading on dispersion of the bacteria. Microbial retention is essential for high rate anaerobic systems. It is important that additional studies focus on microbial retention and contact with the organic wastes since metabolism requires direct contact between the organics and the microbes.

Further studies need to be made on stressed systems to determine which groups of bacteria are the most sensitive and the most easily lost from the system. In these studies butyrate was very easily metabolized to acetate; but the acetate bacteria were the more sensitive under stressed conditions, allowing acetate to be discharged in the treated effluent but very little butyrate was lost. Studies with glucose should focus on the bacteria responsible for metabolizing the propionate which accumulates in stressed systems.

Further studies need to be made on the sulfate reducing bacteria after optimum retention conditions have been established for the methane bacteria. Continuous feeding should be used to determine if there are any significant differences in population dynamics as contrasted with batch-fed systems. It does not appear that a selective toxic agent can be easily found for the sulfate reducing bacteria that does not adversely affect the methane bacteria; but this still is an area of concern that needs further study.

REFERENCES

1. Murray, W.D., and van den Berg, L. 1981. "Effects of Nickel, Cobalt and Molybdenum on Performance of Methanogenic Fixed Film Reactors", Appl.& Envir.Microb. 42:502-05.

2. Schonheit, P., Moll, J., and Thauer, R.K. 1979. "Nickel, Cobalt and Molybdenum Requirement for Growth of Methanobacterium thermoautotrophicum", Arch.Microbiol. 123:105-07.

3. Speece, R.E., Parkin, G.F., and Gallagher, D. 1983. "Nickel Stimulation of Anaerobic Digestion", Water Research 17:677-83.

4. Taylor, B.F., and Oremland, R.S. 1979. "Depletion of Adenosine Triphosphate in Desulfovibrio by Oxyanions of Group VI Elements", Current Microbiol. 3:101-03.

5. Wilson, L.G., and Bandurski, R.S. 1958. "Enzymatic Reactions Involving Sulfate, Sulfite, Selenate and Molybdate", Jour.of Biol. Chem. 233:975-81.

BIOLOGICAL CONVERSION OF GASIFIED MSW INTO METHANE

P. Weaver, A. Frank, R. Gauthier, L. Lundgren,
P. Maness, and S. Lien
Solar Energy Research Institute
Golden, Colorado 80401

ABSTRACT

A large fraction of MSW is strongly resistant to anaerobic digestion, even with long retention times of solids. Thermal gasification can rapidly convert nearly all of the volatile solids of MSW into a relatively homogeneous gas stream consisting primarily of CO and H_2, both of which can be readily metabolized by certain bacteria at ambient temperatures and pressures.

Two hundred thirty-three distinct bacterial strains were isolated which metabolize CO under various conditions. Of these, 84% proved to be photosynthetic bacteria when tested for growth in the light. About 80% of that number, when incubated anaerobically in darkness, performed a water-gas shift reaction, producing H_2 and CO_2 from CO and H_2O with an apparent stoichiometry of 1:1. Rates were in excess of 1 mmol CO shifted per minute per gram cells. The combined H_2 gas streams are ideal substrates for methanogenic bacteria to produce CH_4.

The rate-limiting step in the biological use of gasified MSW is in the mass transfer of the gases from a gas phase into a liquid phase where they can be acted on by bacteria. In order to overcome mass transfer limitations, high concentrations of active bacteria are being reversibly ion exchanged onto electrochemically active electrodes with high surface areas. The electrode-immobilized bacteria will be placed directly within the humid gas stream from a gasifier, free of a bulk liquid phase.

BIOLOGICAL CONVERSION OF GASIFIED MSW INTO METHANE

INTRODUCTION

The major goals of the EMW Program by the year 2000 are to recover 60% of the energy potential of MSW while reducing landfill requirements by 70-80%. For conventional anaerobic digestion, these goals may be difficult to attain. If all of the volatile solids of MSW could be totally digested into CH_4, about three-fourths of the original chemical energy of the MSW would remain in the gaseous product. However, more than half of the volatile solids in MSW are strongly resistant to microbial digestion during reasonable retention times which limits further opportunities for volume reduction and chemical energy recovery.

Thermal gasification of MSW to a low-BTU gas, primarily CO and H_2 (and N_2 in air-blown gasifiers), maximizes volume reduction of the solid waste. Heterogeneous lignocellulosics and other polymers are rapidly converted into fairly homogeneous gaseous products. The gas stream contains about two-thirds of the original chemical energy (plus process heat).

The metabolic activities of some microbes, primarily anaerobic bacteria, are centered around CO and H_2. These microbes have the capacity to metabolize large concentrations of the gases which are toxic to other organisms. Dynatech investigators (1) examined a mixed bacterial system that was capable of growing on and converting CO into CH_4 as an inexpensive alternative to catalytic methanation. Their rates, however, were limited by mass transfer of the gas into a bulk liquid phase and also by low bacterial cell densities.

Our research approach is to reversibly immobilize high concentrations of active bacteria on large surface area support matrices which can be placed directly within a humid gas phase, thereby overcoming mass transfer limitations. The CO component of gasified MSW will be first biologically shifted into $H_2 + CO_2$ and then the entire gas stream will be converted into CH_4 by methanogenic bacteria. The scheme is shown in Fig. 1.

RESULTS AND DISCUSSION

Isolation and Characterization of Bacterial Strains Using CO

Soil and water samples were taken from 44 discrete sites locally. Enrichments were performed on all samples by incubating them under 20% CO for about two weeks in an incomplete medium containing minerals and vitamins (2). Low intensity incandescent light (40 W/m^2) was provided for 12 hours per day. Each culture was then streaked on agar plates to obtain single colonies under aerobic (plates supplemented with 5 g/l sodium malate and 0.5

g/l yeast extract), anaerobic (plates supplemented with 0.5 g/l yeast extract), and photosynthetic (plates supplemented with 5 g/l sodium malate and exposed to 40 W/m^2 incandescent light) conditions under 20% CO. Unique isolates were selected on the basis of colony type, pigmentation and microscopic examination. Duplicate members from the same sample were discarded.

A total of 233 distinct strains were isolated (Table 1). Eighty-five

Table I. CO-Utilizing Strains and Their Photosynthetic Capability

Isolation Condition	No. Strains	No. Photosynthetic Strains
Aerobic	85	49
Anaerobic	61	59
Photosynthetic	87	87
Sum:	233	195

of the strains were isolated from plates incubated aerobically in the dark with CO. Of these, 58% proved to be photosynthetic when subsequently tested for growth in the light. Sixty-one strains were isolated under anaerobic conditions with CO, and all but two could also grow photosynthetically. An additional eighty-seven strains were isolated with photosynthetic conditions. Numerous colonies of green algae were observed photosynthetically with CO, but these were all discarded when they were found to be tolerant of CO but unable to use it. The percentages of photosynthetically competent strains from the aerobic and anaerobic selections may be artificially high. Differences in colony pigmentation were easily recognizable and prompted their selection. Also, since all of the colony selection procedures were performed in air, any strict anaerobes capable of using CO were probably excluded from the final numbers. In all cases, however, each sample yielded at least one pure strain that used CO at rates comparable to, or usually higher than, CO use in the enrichment cultures, so the most active species present were probably isolated.

The presence of photosynthetically competent strains in the aerobic, dark isolates was somewhat surprising. All previous photosynthetic isolates have shown a strong O_2-sensitivity in their CO-metabolizing enzymes as well as a CO-sensitivity to their terminal O_2-respiring enzymes. The new strains may represent a unique metabolic class.

At least 80% of the photosynthetically competent strains, regardless of

how they were isolated, perform a shift of CO + H_2O into H_2 + CO_2 under anaerobic conditions in darkness. The stoichiometry appears to be 1:1 in those examples that have been closely examined. Two types of strains were apparent. Those that grew under anaerobic, dark conditions with CO as sole source of carbon are akin to a strain isolated by Uffen (3), although they may be less nutritionally demanding. The second type, similar to Rhodospirillum rubrum, does not grow under anaerobic, dark conditions, but continues to perform the CO shift reaction. For both types the rates, as yet not optimized, are in excess of one mmol CO shifted per minute per gram cells. Decreases in agitation of the cultures caused decreases in the amount of CO shifted, indicating that mass transfer of the gas into the liquid phase was probably limiting the rates.

Equilibrium conditions starting form 20% CO in the gas phase were reached at 18 ppm CO and 20% H_2 in the one example where it has been examined (i.e., R. rubrum).

Linear rates of CO shift activity were observed for a period of 20 days with a non-growing strain of photosynthetic bacteria in darkness.

A small, downdraft air-blown gasifier operating at 3 liters per minute was constructed with the aid of Tom Reed. Wood pellets were gasified and the gas stream was bubbled through a dark culture of R. rubrum. No ill effects on the bacteria were noted and the CO component of the producer gas was shifted into H_2 at rates comparable to those obtained with pure commercial CO.

Immobilization of Bacteria on Support Matrices

At neutral pH most if not all bacteria appear to have a net negative surface charge due to ionized carboxyl groups. Bacteria adsorb to anion exchange resins such as DEAE-cellulose but not to cation exchange resins such as CM-cellulose. R. rubrum cultures showed no major loss in growth rate when the cells were bound on DEAE-cellulose suspended in liquid medium. A portion of the DEAE-cellulose with adsorbed bacteria could be made to adhere to the glass walls of anaerobe tubes. The rates of CO shift activity in these tubes was significantly enhanced, indicating that there were increased rates of contact of the gaseous CO with the bacteria. Serious drawbacks of using bacteria adsorbed on DEAE-cellulose are the limited increase in exposed surface area and the fact that the ion exchange adsorption is not readily reversible. Neither increased ionic strength nor increased pH caused the release of the bacteria from the resin, a process which is necessary to replace spent bacteria.

Both problems could be overcome with the development of high-surface area, reticulated carbon electrodes that are derivatized with electrochemically active cationic groups. Depending on the porosity of the electrode, 10 cm^3 of the electrode can theoretically bind up to 5 grams of cells in a

surface film one-cell-diameter thick. Electrochemically oxidizing functional groups on the electrode surface will cause a positive surface charge which will electrostatically bind the bacteria. Electrochemically reducing the surface groups to a neutral or negative state should cause the release of the bacteria.

Methylthiophene was electrodeposited onto reticulated carbon electrodes. No stable bacterial cell association was observed with the oxidized polymethylthiophene coating. This may have been due to organic material in the bacteria reducing the highly oxidizing polymer.

Polypyrrole coatings were deposited on tin oxide electrodes. Bacteria could be co-deposited on the electrode surfaces in thin films. However, the binding was not reversible.

Vinylferrocene was also electropolymerized on tin oxide electrodes. In this instance the oxidized polymer rapidly adsorbed bacteria from a liquid suspension. The bacteria remained firmly attached to the electrode when it was physically removed from the suspension and rinsed in water. Upon reduction of the polymer to a neutral charge state a significant fraction of the bacteria were released back into solution.

FUTURE WORK

Although we have a basic system that will allow us to test the concept of performing a gas phase, microbial conversion of gasified MSW into CH_4, several improvements need to be made. Additional redox polymers need to be synthesized and examined in order to increase the rates and extents of reversible bacterial binding. The physical requirements for bacteria immobilized in a gas phase will be determined as they affect rates, yields and durabilities. Integrated tests will be performed using pure gases and gasified MSW at bench-scale levels.

REFERENCES

1) Tracy, C. A., and Ashare, E. 1981. Dynatech R/D Final Report on SERI Contract No. XB-9-8356-1. Report No. 2125. Cambridge, Mass.

2) Weaver, P. F., Wall, J. D., and Gest, H. 1975. Arch. Microbiol. 105, 207-16.

3) Uffen, R. L. 1976. Proc. Nat. Acad. Sci. 73, 3298.

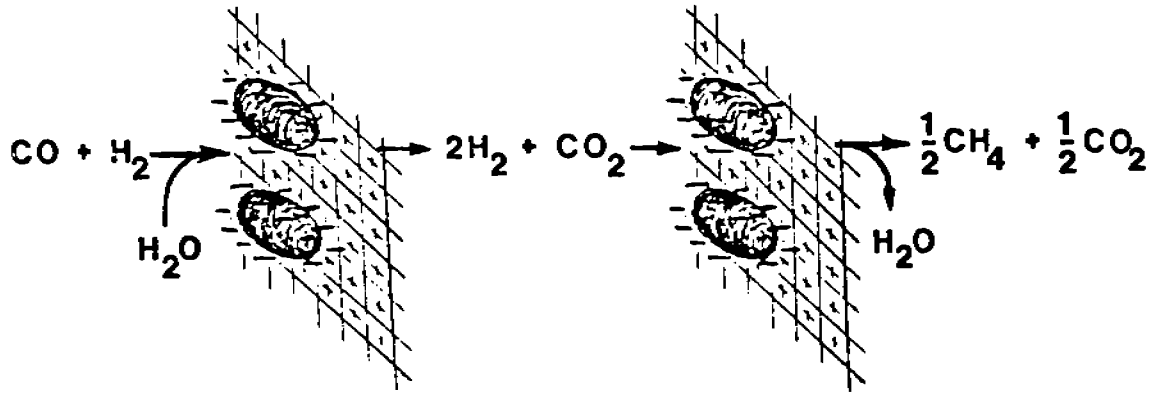


Fig. 1: Scheme for converting gasified MSW into methane using immobilized bacteria free of a bulk liquid phase.

USE OF MONOCLONAL ANTIBODY PROBES TO TRACK EXTERNAL ADDITIONS OF IMPROVED HYDROLYTIC MICROORGANISMS IN A MUNICIPAL SOLID WASTE ANAEROBIC DIGESTER

Everly Conway de Macario and Alberto Macario
Wadsworth Center for Laboratories and Research
New York State Department of Health
School of Public Health Sciences
State University of New York at Albany
Albany, New York 12201

and

Christopher J. Rivard and Karel Grohmann
Biotechnology Research Branch
Solar Energy Research Institute
Golden, Colorado 80401

ABSTRACT

Several new anaerobic microorganisms which have improved hydrolytic enzymatic activity, specifically cellulose degrading ability, have been isolated and characterized in the literature. These microbes are of the genus Clostridium and are not the predominant hydrolytic microorganisms in manure digestion systems. The development of a real-time tracking system employing monoclonal antibodies to monitor the external addition of one such microbe, Clostridium populeti, to a standard laboratory scale anaerobic digester is underway. This target microbe has been grown in mass culture and harvested for antibody probe development as well as preserved frozen for later laboratory digester addition studies. The antigenicity of the target microorganism, Cl. populeti is currently being tested in laboratory animals. Rabbits and mice were immunized to determine immunogenicity and, eventually, to prepare monoclonal antibody probes. Assays and probes previously developed for studying methanogens were used to study Cl. populeti to determine their applicability to this non-methanogen and to establish whether crossreactions occur. Discussions to coordinate tasks and set forth priorities were entertained and the purchase of instruments and reagents necessary to implement the project was undertaken. Cl. populeti was found to be amenable to immunologic study using the strategies and methods already standardized in our laboratory.

Note: Subcontract funding for (Conway de Macario) began August 20, 1987.

USE OF MONOCLONAL ANTIBODY PROBES TO TRACK EXTERNAL ADDITIONS OF IMPROVED HYDROLYTIC MICROORGANISMS IN A MUNICIPAL SOLID WASTE ANAEROBIC DIGESTER

INTRODUCTION

The use of anaerobic digestion for municipal solid waste (MSW) disposal is desirable from many perspectives. The anaerobic digestion process produces significant amounts of energy (methane) and is considerably less energy intensive than aerobic processes which require high agitation rates to achieve sufficient aeration (Bryant 1979, Gujer and Zehnder 1983). The anaerobic process also accomplishes the reduction in the waste bulk with less microbial biomass buildup than aerobic processes.

The process has been considered slow, however, requiring 10-30 days for substantial digestion of polymeric sugar feedstocks such as cellulose found in municipal solid wastes. The limiting or slow step in the anaerobic digestion process is the hydrolysis of the sugar polymers to monomeric sugars (Boone 1982).

In recent years, new anaerobic microorganisms have been isolated and described possessing greater apparent hydrolytic enzyme production rates than those microbes usually found in anaerobic digestion systems (Sleat et al 1984, Sleat and Mah 1985). The reason that these improved microbes are not found in high numbers in most digestion systems is unknown. It is therefore imperative that the addition and competition of these new microbes be studied in a functioning anaerobic digester system fed municipal solid waste, a high cellulose feedstock. Until recently, a fast, real-time analysis method for tracking added microorganisms was not available. Monoclonal antibodies have been used as probes in pure cultures and complex systems, such as digester sludges, to track specific microbes (Macario and Conway de Macario 1982, 1986). This powerful tool can be used to determine not only relative numbers of cells, but also placement within flocks of cells or attachment of cells to material within complex systems. This allows the researcher more insight into the competition of the added microorganisms than is available from the standard plating or roll tube assessment of viable cell numbers. The later method often takes several weeks to determine colony numbers and thus changes in numbers of microbes in the digestion system.

The incorporation of microorganisms with greater hydrolytic enzyme activity is required to effect an increase in the overall anaerobic digestion rate for the system. Faster reaction rates would allow for shorter retention times for the digester and improve the economics of the process dramatically.

MATERIALS AND METHODS

Microorganisms

Strains of Clostridium populeti and Cl. cellulovorans were provided as a gift by Dr. Robert Mah, University of California at Los Angeles. Strains of Clostridium thermocellum, and Cl. acetobutylicum were obtained from the American Type Culture Collection (ATCC), Rockville, Maryland.

Culture Media

The Clostridium populeti and cellulovorans were maintained on 2% agar (Difco, Detroit, MI) slants of the standard medium as described (Sleat et al 1984, Sleat and Mah 1985) using standard anaerobic techniques (Hungate 1950). Mass cultures of 250, 500, and 2500 ml were prepared using round bottom boiling flasks with total volumes 500, 1000, and 5000 ml respectively. After gassing the head space the flasks were closed by wiring on rubber stoppers. After autoclaving the media was allowed to cool, the appropriate volume of sterile sulfide/carbonate solution was added using a syringe and needle to the flasks using aseptic techniques.

Microscopy

Observation of microbial cultures were conducted using wet-mount slide preparations using a Nikon Labophot microscope equipped with phase contrast illumination at 1000x power. Documentation of wet-mount observations was obtained using a Nikon automatic exposure camera system installed on the Nikon microscope.

Preservation of Microorganisms

Cultures preserved for use in immunological specificity testing were taken from 50 or 250 ml cultures. When the culture had reached an approximate optical density of 1-2, the culture was harvested by centrifugation under aerobic conditions at 4°C, in 50 Corex centrifuge tubes using a Sorvall model RC-5B centrifuge and SS-34 rotor at 7,000 rpm for 20 minutes. The supernatant was discarded and the cell pellet resuspended in an appropriate volume of 4% formalin/0.85% sodium chloride to obtain an optical density of approximately 2. The cell suspension was pelleted again and resuspended in the fixing solution as before. The preserved cells were then stored at 4°C until shipment to the Macario laboratory for use in specificity testing.

Mass cultures (2.5 liters) of Clostridium populeti, to be used in future digester addition studies, were harvested using a Sorvall model RC-5B centrifuge in 500 ml plastic bottles with a GSA rotor. The exposure of the culture with oxygen was minimized by outgassing of the centrifuge bottles with oxygen-free nitrogen gas. The cells were centrifuged at 6,000 rpm at 4°C for 30 minutes. The supernatant was discarded and the cell pellets combined and frozen in one 50 ml corex centrifuge tube under a nitrogen gas head space and stored at -70°C using a Revco ultralow freezer until use.

Immunological Antigenicity Testing in Laboratory Animals

Poly- and mono-clonal antibody probes are prepared as described previously (Macario and Conway de Macario 1985a). The probes' specificity spectra are determined using the immunizing and other relevant antigens and the molecular specificities of monoclonal antibody probes are determined by inhibition-blocking, direct-binding, and other assays as described (Macario and Conway de Macario 1982, 1985a). Identification of microbes in pure cultures and in mixtures (e.g. digester samples) is done by antigenic fingerprinting applying the slide immunoenzymatic assay (SIA) constellation, including indirect immunofluorescence and auxiliary procedures (Macario and Conway de Macario 1982, 1985a, 1985b, 1986). These methods are also applied for quantifying

each bacterial strain (immunotype) (Macario and Conway de Macario 1987).

RESULTS

Mass cultures of the target hydrolytic microorganism, Clostridium populeti were prepared. Approximately 20 grams of wet packed cells were frozen and are to be used in future addition studies to laboratory anaerobic digesters fed municipal solid waste. Cultures of Cl. populeti were also preserved as described above and used in the initial stages of antigenicity testing using laboratory animals. Rabbits and mice were inoculated with Cl. populeti to test immunogenicity and eventually to generate poly-and mono-clonal probes. The animals did not show any sign of disease, indicating that problems derived from possible toxic effects of Cl. populeti are not to be expected. This observation is very encouraging. Sera from the inoculated animals will be collected as soon as the immunization schedules are completed. In the meantime, minimal quantities of sera are being obtained to monitor the antibody response. Preliminary data indicate that antibodies are being produced by the animals.

Several strains of microorganisms which may be closely related to Cl. populeti, and thereby result in cross-reactivity with the antibody probe were cultured and preserved for specificity testing of the monoclonal antibody probe to be developed. These bacteria are of the genus Clostridium and include Cl. cellulovorans, thermocellum, and acetobutylicum.

DISCUSSION/FURTHER WORK

Work was begun to produce monoclonal antibody probes for Cl. populeti. Preliminary results indicate that this microbe is amenable to immunologic analysis. Means for immunologic identification and quantification, and eventually for manipulation of Cl. populeti should become available as a result of implementing this project.

Immediate objectives are to complete the immunization of rabbits and mice, determine immunogenicity and antigenicity of Cl. populeti, obtain antisera, and construct hybridomas.

During a second year, mono-clonal antibodies should be produced in larger quantities and characterized.

Availability of monoclonal antibody probes for Cl. populeti will allow tracking this species in digesters, along with methanogens (for which probes are already available), and along with other microbes. The information obtained should provide the basis for deciding which organisms should be included when biologically designing a digester.

REFERENCES

Boone, D.R., 1982. "Terminal Reactions in the Anaerobic Digestion of Animal Waste," Appl. Environ. Microbiol. 43:57-64.

Bryant, M.P., 1979. "Microbial Methane Production -- Theoretical Aspects," J. Animal Science 48:193-201.

Gujer, W., and Zehnder, A.J.B. 1983. "Conversion Processes in Anaerobic Digestion," Water Science Tech. 15:127-167.

Hungate, R.E., 1950. "The Anaerobic Mesophilic Cellulolytic Bacteria," Bacteriol. Rev. 14:1-49.

Macario, A.J.L., and Conway de Macario, E. 1982. "The Immunology of Methanogens: A New Development in Microbial Biotechnology," Immunol. Today 3:279-284.

Macario, A.J.L., and Conway de Macario, E. 1985a. "Monoclonal Antibodies of Predefined Molecular Specificity for Identification and Classification of Methanogens and for Probing Their Ecologic Niches," pp. 213-247. In: A.J.L. Macario and E. Conway de Macario (eds.). Monoclonal Antibodies against bacteria, Academic Press, Inc., Orlando Florida, Vol. II.

Macario, A.J.L., and Conway de Macario, E. 1985b. "Antibodies for Methanogenic Biotechnology," Trends Biotechnol. 3:204-208.

Macario, A.J.L., and Conway de Macario, E. 1986. "Methanogens in Digesters: Identification and Quantification in Microsamples by Direct Testing with Antibody Probes," pp. 1009-1020. In: Energy from Biomass and Wastes X. Institute of Gas Technology, Chicago, Illinois - Elsevier Applied Science Publishers Ltd., Barking, Essex, England.

Macario, A.J.L., and Conway de Macario, E. 1987. "Quantitative Immunologic Analysis of the Methanogenic Flora of Digesters Reveals Considerable Diversity," Appl. Environ. Microbiol. (In press).

Sleat, R. and Mah, R.A. 1985. "Clostridium populeti sp.nov., a Cellulolytic Species from a Woody-Biomass Digester," Int. J. Syst. Bacteriol. 35:160-163.

Sleat, R., Mah, R.A., and Robinson, R. 1984. "Isolation and Characterization of an Anaerobic, Cellulolytic Bacterium Clostridium cellulovorans sp.nov.," Appl. Environ. Microbiol. 48:88-93.

Process Engineering Experiments

OPERATION OF AN EXPERIMENTAL TEST UNIT FOR THE BIOCONVERSION OF WASTE AND BIOMASS TO METHANE

Richard Biljetina
Institute of Gas Technology
3424 S. State Street
Chicago, Illinois 60616

INTRODUCTION

The Gas Research Institute (GRI) has been the primary sponsor of a research program at the Community Waste Research Facility (CWRF) located at the Walt Disney World Resort Complex. Four institutions have teamed to provide solutions to community waste treatment and disposal problems. They are: Walt Disney Imagineering; University of Florida — Agricultural Engineering; Reynolds, Smith and Hills; and the Institute of Gas Technology (IGT). Of primary importance to this research effort is the implementation of low-cost, nonenergy-intensive waste treatment and recovery technologies and the net production of energy (methane) from biomass and waste resources. The production of methane is being studied at the CWRF in a biogasification experimental test unit (ETU) designed, constructed, and operated by IGT (Figure 1).

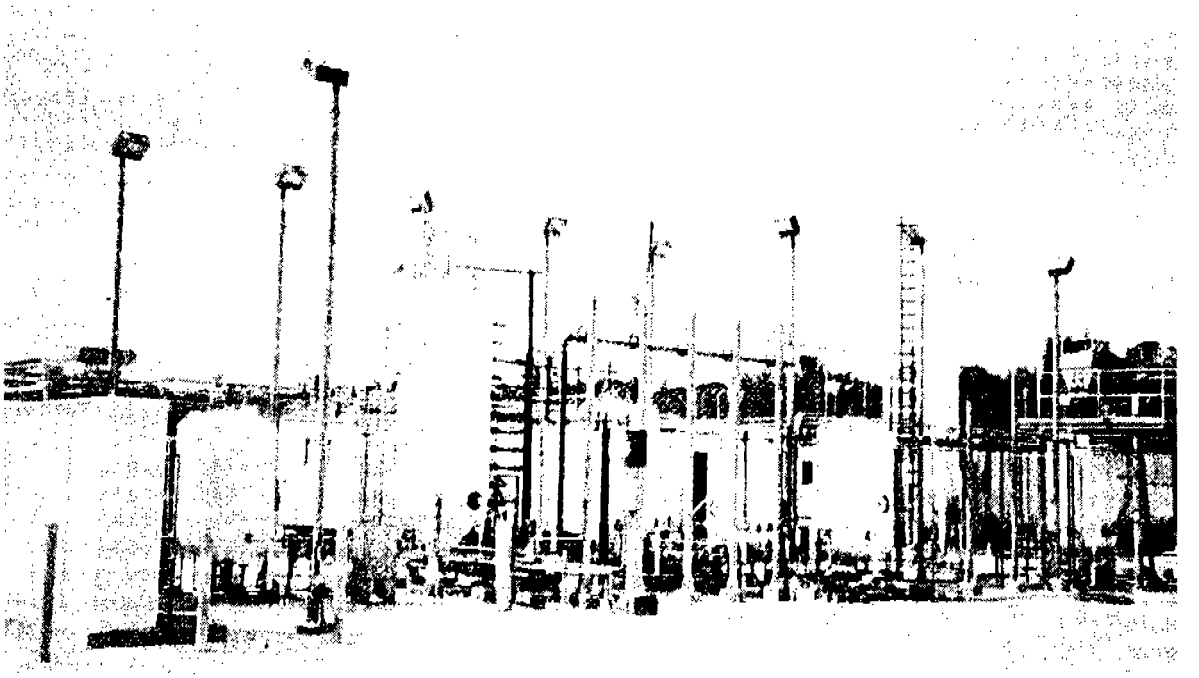


Figure 1. EXPERIMENTAL TEST UNIT

OBJECTIVE

The objective of the ETU is to demonstrate biogasification concepts for the production of methane from biomass and wastes and to obtain data for commercial scale-up. The design of the conversion system incorporates a high degree of flexibility to allow testing with a number of different feedstocks and includes front-end biomass processing and slurry preparation equipment, a cold flow test column, a 1200-gallon digestion unit, digester effluent processing equipment, and gas handling equipment. The primary function of this unit is to validate baseline digester operation and performance data obtained in the laboratory and to evaluate larger scale equipment for material preparation, transport, and unit operations. Results obtained from the ETU provide the basis for complete conceptual process designs and cost estimates for process demonstration units.

APPROACH

Operation of the ETU digester began in January of 1984. Initial tests were conducted on blends of water hyacinth and sludge to support a process concept that provides effective secondary and tertiary wastewater treatment using aquatic macrophytes such as water hyacinths. The water hyacinths were harvested from five quarter-acre treatment channels at the CWRF and were mixed with sludge from the primary clarifier serving the Walt Disney World Resort Complex. Blend ratios were maintained between 2:1 and 1:1 water hyacinth/sludge to simulate expected production quantities from larger commercial treatment facilities. Anaerobic digestion was performed in an unmixed, solids concentrating (SOLCON) digester which is the main component of the ETU facility. Data was collected during six different performance periods over a 2-year period of uninterrupted digester operation.[1] The ETU digester consistently out-performed a sentinel 50-liter continuous stirred tank reactor (CSTR) operated at the same conditions. Methane yields were between 10% to 30% higher in the ETU digester. System studies indicated that the combined wastewater treatment and biogasification facility could produce methane at less than $2.00 per MM Btu.[2]

Following completion of the test program on water hyacinth/sludge blends, preparations were made at the ETU to feed an agricultural product, sorghum, to be followed by tests on municipal solid waste.

Sorghum represents a potentially large biomass resource for conversion to methane. A total of 17.8 million acres of sorghum were planted in the U.S. in 1985. Research at Texas A&M indicates that yields up to 36 tons (fresh weight) of sorghum are possible for sweet sorghum grown for biomass production. In 1986, two 6-month tests were conducted on sorghum. The first test was conducted with an Atx623xRio sorghum at mesophilic conditions, and the second was conducted with an MN-1500 sorghum at thermophilic conditions. The ETU methane yields exceeded those of the sentinel CSTR by 50% and 20%.[3] Although we would recommend additional investigations on sorghum at thermophilic conditions, sufficient data were collected to allow scale-up of a solids-concentrating digester for the conversion of sorghum to methane.

In 1987, conversion studies were begun using the refuse-derived-fuel (RDF) fraction of municipal solid waste. The RDF is obtained from a resource recovery plant located in Baltimore, Maryland. The plant was chosen as a source because it is capable of delivering a 1/2 x 0 inch sized feed material to the ETU and eliminates the need for installation of additional sizing and separation equipment at the ETU.

An event schedule is given in Table 1. The planned program thus provides a digestion data base for the three major waste resources generated in the U.S.: sludge and biomass from wastewater treatment, municipal solid wastes, and agricultural byproducts.

Table 1. ETU OPERATING SCHEDULE

Start-Up and Digester Acclimation	January-June 1984
Water Hyacinth/Sludge Conversion Tests	July 1984-December 1985
Sorghum Conversion Tests	January 1986-December 1986
MSW Conversion Tests	January 1987-June 1988

ETU DESCRIPTION

Figure 2 provides a schematic diagram diagram of the biogasification facility. Three main processing areas can be identified.

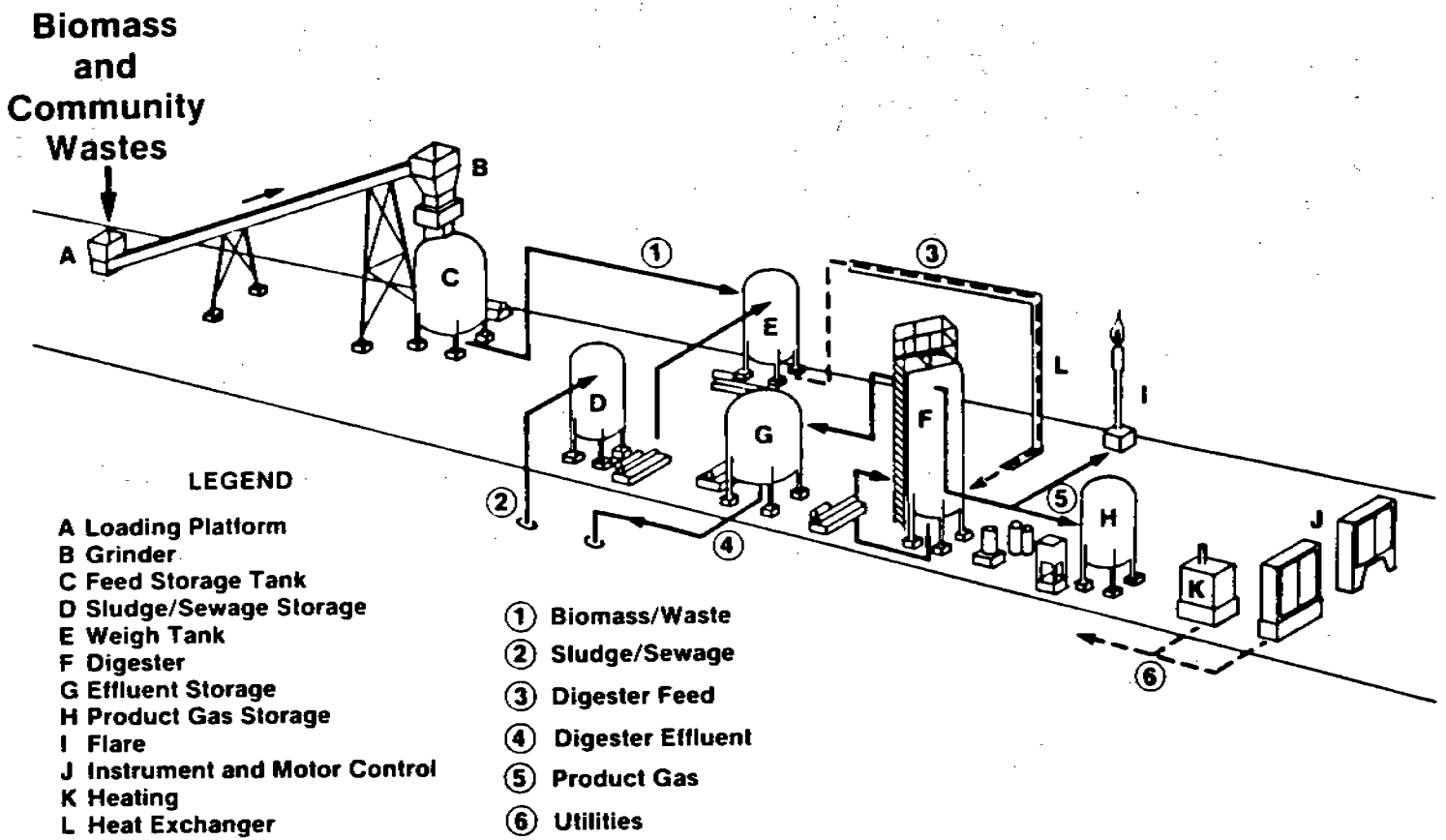


Figure 2. SCHEMATIC DIAGRAM OF THE ETU FACILITY

Feed and Slurry Preparation

The ETU is designed to fine-grind any biomass and solid waste prior to slurry preparation. Material is sized down to 1/8 inch and diluted (if necessary) to 5 to 10 weight percent solids in order to allow transport by progressing-cavity pumps in 2-inch lines. The feed slurry storage tank (C) is insulated and cooled with well-water (70°F) to reduce degradation of feedstocks prior to digestion. Both a tank mixer and the discharge pump can be used to keep the solids in suspension. Feed is prepared two to three times a week and requires 2 to 4 hours of one operator's time.

Water hyacinths were harvested from the treatment channels and first pre-chopped to about 2 inches at the loading platform. Sorghum was obtained prechopped from Texas A&M University and was stored in lined drums. The Atx623xRio sorghum was ensiled for about 1 year prior to digestion; the MN-1500 was ensiled about 6 weeks prior to digestion. The RDF was obtained prechopped from the National Ecology of Baltimore and is being stored in lined drums housed in a refrigerated trailer.

The water hyacinth, after grinding, did not require addition of dilution liquid to attain a pumpable slurry. An energy evaluation indicated that less than 2% of the plant energy production (methane equivalent) is required for the chopping and grinding operation. Both the sorghum and the RDF require dilution. Raw sewage pumped underground from the existing treatment plant and digester effluent recycle liquor are being used to prepare slurries between 5 to 10 weight percent. Slurry inventories in the storage tanks are monitored routinely for changes in composition and temperature. Typically, 5 to 10 weight percent of the feed material does convert to organic acids during the intermediate storage period. Sorghum slurries contained a high organic acid content (about 20 weight percent) due to prior ensiling. This then required special analytical techniques to ensure complete characterization of the feed to the digester and to allow good material balance closures.

Feed Blending and Weighing

A weigh tank (E) mounted on load cells allows accurate measurement of blend quantities, and most important, it provides a highly accurate record of the daily feed quantities added to the digester. Repeated calbirations have shown that the system maintains weighing accuracies over its operating range

of ±1%. The system is designed to feed up to 2000 lb/day of slurry to the digester. The reliability of this weighing system has been a key element in obtaining better than 100% ±10% material balance closures around the digester for all but one of the steady-state data acquisition periods.

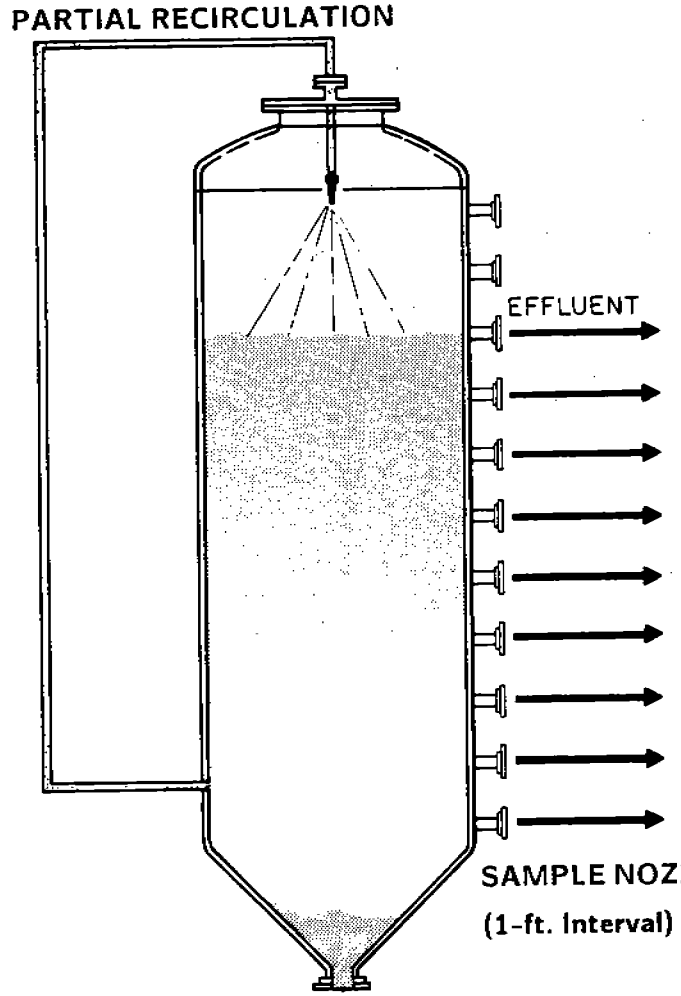
Digestion and Gas Handling

Biogasification of the biomass and wastes occurs in a 1200-gallon, unmixed anaearobic digester (F) having a height-to-diameter ratio of 2:1. The unit can be modified to alternative reaction systems. For instance, internal rings have been provided to allow attachment of baffles, and provisions for both hydraulic and gas mixing are included. The digester is fully jacketed and insulated to allow automatic temperature control of the contents. Twelve thermocouples monitor internal temperatures and 2-inch ports are provided every foot of vertical height to allow sampling and flexibility in the selection of feed, discharge, and recirculation points.

Each of the feedstocks tested to date, although similar in chemical composition (Table 2), exhibited a different solids distribution pattern within the digester. In addition, data on volatile acids and pH profiles in the digester helped in the selection of feed, discharge, and recirculation points. Figure 3 provides the solids profiles obtained in the digester at similar loading rates for water hyacinth/sludge blends, sorghum, and RDF. On the basis of these data, upflow operation was chosen for the sorghum material and downflow appears to be the best mode of operation for water hyacinth/sludge and RDF feedstocks. These effects were not readily observed in laboratory-scale digesters, therefore stressing the importance of following a logical progression of scale-up toward commercial operations.

RESULTS

The bioconversion of sludge and water hyacinth blends in the ETU digester was highly successful. Six tests were completed between July 1984 and December 1985. Performance data were collected in both the upflow and downflow mode of operation with water hyacinth sludge blends of 2:1 and 1:1, respectively. Temperatures were maintained at mesophilic conditions (95°F), and loading rates were controlled at 0.2 lb VS/ft^3-day (11-day hydraulic retention time) except for the last test, which was conducted at a loading rate of 0.3 lb VS/ft^3-day (7-day hydraulic retention time). Operation during the entire test period was uninterrupted; no nutrient addition was necessary.



	2.1 Water Hyacinth Sludge Blend	Sorghum	MSW
	7.6	2.2	10.2
	6.2	1.7	4.1
	6.8	1.8	7.5
	6.7	2.1	3.5
	6.8	2.1	8.1
	5.2	2.7	3.0
	0.8	3.9	3.7
	0.7	5.5	2.2
	1.5	5.9	2.4

Figure 3. SOLIDS PROFILES IN THE DIGESTER FOR VARIOUS FEEDSTOCKS AT SIMILAR LOADING RATES (0./2 lb VS/ft^3-day) and HRT's (11-13 days) MEASURED IN WEIGHT PERCENT TOTAL SOLIDS

Table 2. PHYSICAL AND CHEMICAL CHARACTERISTICS OF ETU FEEDSTOCKS

Feed Type (As-Received)	Primary Sludge	Hyacinth	Sorghum	MSW**
Total Solids TS, % net wt	4.8	4.9	27.5	93.3
Volatile Solids, VS % TS	83.6	82.9	93.8	91.1
Elements, % TS				
Carbon, C	47.1	40.7	44.4	41.9
Hydrogen, H	7.04	5.72	6.16	5.67
Nitrogen, N	3.75	3.02	1.15	0.59
Phosphorus, P	0.56	0.73	0.24	0.05
Sulfur, S	0.49	0.76	0.10	0.12
Heating Value, Btu/lb (VS)	11,000	8,370	8,160	7.320
Recoverable Energy in Product Gas,* Btu/lb VS	10,000	5,000	6,000	4,000

**As determined by ABP assays. These data are accurate to within ±10%.

** RDF fraction.

The digester is not mixed to maximize the solids retention time. Partial recirculation is practiced to maintain an active culture at the top of the digester. Gas yields as high as 12.4 SCF/lb VS and methane yields as high as 7.9 SCF/lb VS (7.2 lb organic matter) added were obtained using a 1:1 water hyacinth/sludge blend. This represents over 90% of the maximum expected biodegradable yield as measured by bioassays for the water hyacinth and sludge material. The methane content of the gas varied between 59 and 64 volume percent. Table 3 provides an overview of the test results. In all cases the ETU methane yields exceeded those of a sentinel 50-liter conventional stirred tank reactor operated in the field receiving the same feed material as the ETU.

Data collected on sorghum in the ETU during 1986 verified earlier observations by Texas A&M that this is an excellent material for conversion to methane. Methane yields of 5.3 SCF/lb organic matter fed were obtained at a loading rate of 0.27 lb of organic matter/ft^3-day. This represents nearly complete conversion of the biodegradable portion of the sorghum and exceeds the yields obtained in a conventional CSTR by 50%.

Table 3. ETU DIGESTER TESTS FOR WATER HYACINTH/SLUDGE BLENDS

Test Number	1	2	3	4	5	6
Duration, months	5	3	3	4	1	3
Steady-State Period, weeks	4	3	4	4	4	4
Operating Mode, flow	Up	Up	Down	Down	Down	Down
Blend Ratio, WH/SL	2:1	1:1	1:1	2:1	2:1	2:1
Temperature, °F	95	95	95	95	95	95
Loading Rate, lb org. matter/ft^3-day	0.2	0.2	0.2	0.2	0.2	0.3
HRT, days	12	11	11	11	10	7
ETU Methane Yield, SCF/lb-organic matter	4.2	6.4	7.2	5.8	5.5	4.1
ETU Methane Production Rate vol/vol-cult.-day	0.9	1.3	1.5	1.3	1.2	1.5
CSTR Methane Yield, SCF/lb-organic matter	3.5	5.9	6.5	4.4	4.3	3.8
ETU Carbon Balance, wt %	98	105	103	108	99	94

At thermophilic conditions, methane yields of 4.4 SCF/lb organic matter fed were obtained for a loading rate of 0.47 lb of organic matter/ft^3-day and methane production was increased from 1.4 to 2.0 vol/vol-cult.-day (Table 4). With further optimization it may be possible to increase the methane yields even more at thermophilic conditions in the solids-concentrating digester. Operation once again was uninterrupted; however, nutrient addition was necessary to bring the C/N, C/P, and C/S ratios in the feed to 15:1, 100:1, and 150:1, respectively.

The test program on RDF feeds was initiated in late 1986. Solids distribution and slurry rheology were first studied in a 25-foot-high, 1000-gallon cold-flow test column. As a result of these studies, modification of existing equipment was minimized and downflow operation was chosen for the RDF feed material in the ETU digester. Figure 4 provides an overview of the production parameters in the ETU digester since February of 1987. The first test period was completed in June. The RDF is mixed with primary sludge at a ratio of 15:1, the expected community waste ratio. If no digester effluent is recycled as planned for the first test, addition of nutrients is necessary. Table 5 provides average values for the RDF/sludge blend fed to the digester

Table 4. ETU DIGESTER TESTS FOR ENSILED SORGHUM

Test Number	1	2
Duration, months	6	6
Steady-State Period, weeks	4	4
Operating Mode, flow	Up	Up
Temperature, °F	94	132
Loading Rate, lb organic matter/ft^3-day	0.25	0.47
HRT, days	13	10
ETU Methane Yield, SCF/lb-organic matter	5.3	4.4
ETU Methane Production Rate vol/vol-cult.-day	1.4	2.0
CSTR Methane Yield, SCF/lb-organic matter	3.6	3.7
ETU Carbon Balance, wt %	93	97

Table 5. RDF/SLUDGE BLEND FEED QUALITY AS FED TO THE DIGESTER FOR THE FIRST TEST PERIOD

Total Solids, wt %	7.1
Volatile Solids, wt % TS	74.5
Solids Ratio, lb RDF TS/lb PS TS	15:1
Volatile Acids, mg/L as acetic	7200
Ammonia Nitrogen, mg N/L	1230
Total Alkalinity, mg/L as $CaCO_3$	5800
pH	6.0
Added NH_4Cl, mg/L	5640
Added KH_2PO_4, mg/L	1100
Added Na_2S, mg/L	260
Added $NaHCO_3$, mg/L	7600
Nutrient Ratios	
C/N	15:1
C/P	100:1
C/S	150:1

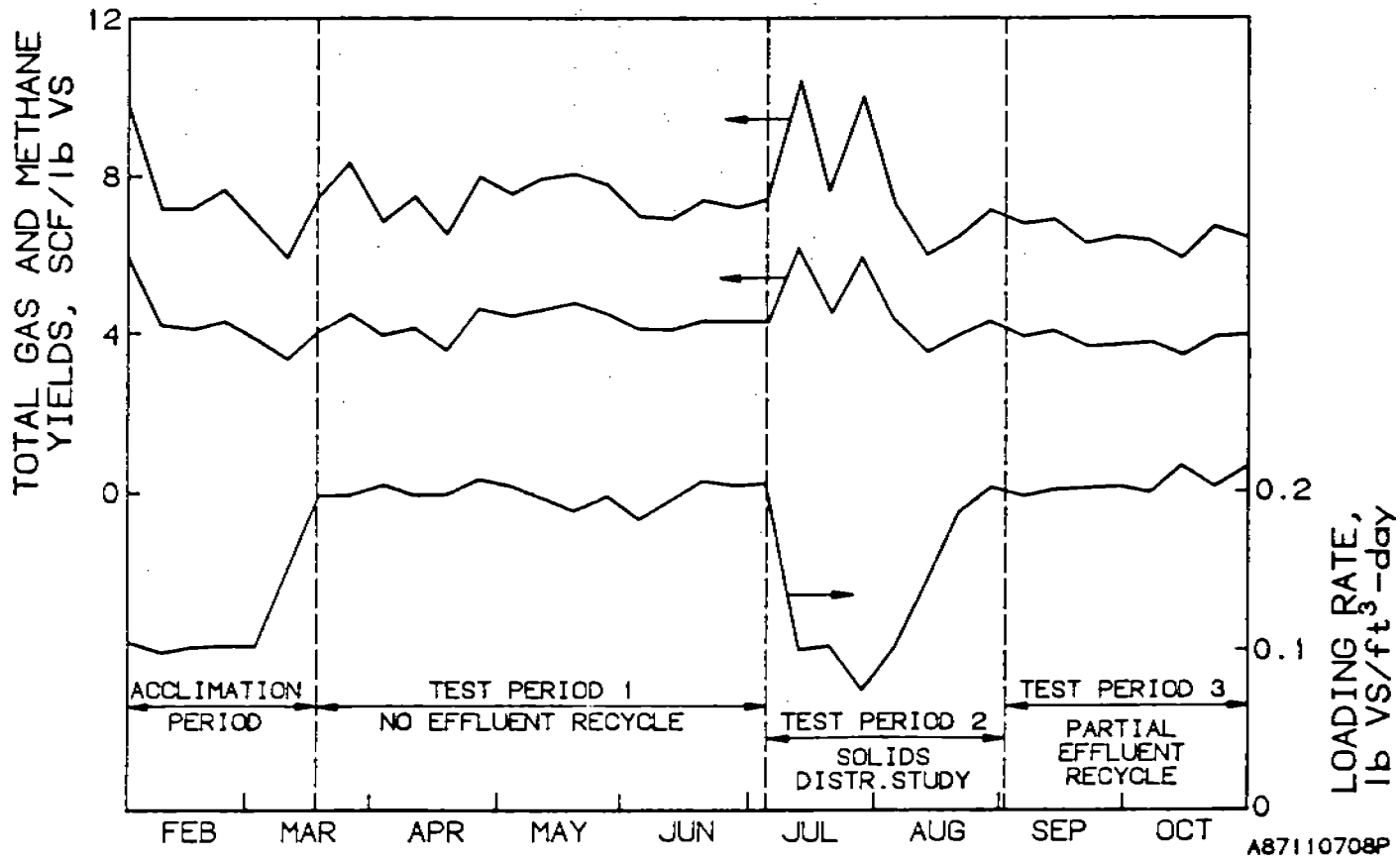


Figure 4. ETU DIGESTER PERFORMANCE WITH RDF FEED (1987)

during the first test period. A second test (Period 3) is in progress with partial effluent recycle. This has cut the need for nutrient addition in proportion to the recycle stream. Further tests are planned with complete effluent recycle, which should eliminate the need to add any nutrients to the RDF feed material.

Table 6 is a data summary for the steady-state data acquisition during Period 1. The results have been extremely encouraging. The ETU digester operated at mesophilic conditions is achieving methane yields that exceed both mesophilic and thermophilic sentinel CSTR digesters operated on the same feed material. The ETU digester is consistently producing a methane yield that exceeds 90% of the maximum biodegradable yield as measured by bioassay. Table 7 provides a comparison of the yields observed for the first test period. These data are being further confirmed during the second test period.

SUMMARY

The data collected at the ETU for three different feed materials indicate that an unmixed, solids concentrating (SOLCON) digester consistently outperforms a conventional CSTR, operating on feed slurries containing 5 to 10 weight percent solids. Methane yields have exceeded a CSTR digester in all cases by 10% to 50%. This type of digestion system is also extremely stable and flexible. Over 3-1/2 years of uninterrupted operation have been logged in the digester. During that time feedstocks, loading rates, and temperatures were changed without the need to reinoculate a "stuck" digester. Transition periods for different feedstocks and loading rates have been very smooth. Even the transition from mesophilic to thermophilic was uneventful. However, transitions from thermophilic operation back to mesophilic operation will require longer periods and special operating techniques to avoid a "stuck" digester.

Following the operating campaign on sorghum, the digester culture was transferred for 1 day to a holding tank to allow inspection of the digester. Despite the unmixed operation, no unusual solids accumulations or "dead zones" were observed. All internals were in excellent condition.

Table 6. ETU PERFORMANCE DATA SUMMARY

Performance Period	9
Date	5/18-6/21
Duration, Steady-State Data Acquisition	5 weeks
Feed Components Characteristics	
RDF Solids Content, wt %	79.0
RDF Volatile Solids Content, wt % TS	85.6
Primary Sludge Solids Content, wt %	3.62
Primary Sludge Volatile Solids Content, wt % TS	90.3
Primary Sludge Volatile Acids Content mg/L acetic	1200
Digester Blend Feed Characteristics[a]	
Daily Loading Rate, lb OM/ft^3[b]	0.23
Daily Loading Rate, lb VS/ft^3	0.20
Solids Content, wt %	7.07
Volatile Solids Content, wt % TS	74.5
Volatile Acids Content, mg/L acetic	7300
pH	6.0
Alkalinity, mg/L $CaCO_3$	5800
NH_3-N Content, mg/L	1000
$NaHCO_3$ Added, lb/lb organic matter fed	0.15
NH_4Cl Added, lb/lb organic matter fed	0.11
KH_2PO_4 Added, lb/lb organic matter fed	0.022
Na_2S Added, lb/lb organic matter fed	0.008
Digester Operating Conditions	
Feed Injection	Top
Daily Loading Frequency	Once
Culture Volume, ft^3	137
Temperature, °F	94.4
pH[c]	6.7-7.4
Volatile Acid Content, mg/L acetic[c]	0-3400
Daily Culture Recirculation Time, h	4
HRT, days	16
Number of HRT's Completed	6
Performance Data	
Daily Gas Production, SCF	205
Total Gas Yield, SCF/lb OM	6.6
Methane Content, vol %	59.6
Methane Yield, SCF/lb OM	3.9
Methane Yield, SCF/lb VS	4.4
Percent of Maximum Yield[d]	97
Methane Production Rate, vol./vol.-cult.-day	0.9
Carbon Conversion, wt %	37.1
Carbon Balance, wt %	115
Effluent Characteristics	
Effluent withdrawal, nozzle number	4-7 Bottom
Solids Content, wt %	6.18
Volatile Solids Content, wt % TS	66.3
Volatile Acids Content, mg/L acetic	400
pH	7.2
Alkalinity, mg/L $CaCO_3$	5900
NH_3-N Content, mg/L	800

[a] Organic matter (OM) consists of volatile solids and 90% of the volatile acids.

[b] 15:1 Blend of RDF/sludge.

[c] Varies with digester height.

[d] Maximum yield was determined by 60-day ABP assay.

Table 7. METHANE YIELD COMPARISON FOR BALTIMORE RDF

	Methane Yield SCF/lb OM added
ABP Assay (maximum expected yield)	4.0
ETU Digester (mesophilic operation)	3.9
CSTR Digester (thermophilic operation)	3.1
CSTR Digester (mesophilic operation)	2.9

ACKNOWLEDGMENT

We wish to acknowledge the support and assistance received from the personnel at Walt Disney Imagineering, University of Florida, Texas A&M University, and our field scientists in the operation of the ETU facility. We also gratefully acknowledge our sponsors, the Gas Research Institute, Southern California Edison, and the U.S. Department of Energy.

REFERENCES CITED

1. Biljetina, R., Chynoweth, D. P., Srivastava, V. J., Janulis, J. A., Razik, A. and Smallwood, S., "Biogasification of Walt Disney World Biomass Waste Blend." Final Report for Gas Research Institute, Contract 5082-223-0567, October 1986.

2. Black and Veatch, Engineers-Architects, "An Economic Systems Assessment of the Concept of a Water Hyacinth Wastewater Treatment/Methane Production." Final Report for Gas Research Institute, Contract No. 5082-511-0629, 1983.

3. Biljetina, R., Srivastava, V. J., Fannin, K. F., Janulis, J. A. and Henry, M. P., "ETU Program for the Production of Methane from Community-Derived Wastes." Annual Report for Walt Disney Imagineering, Contract No. 9MP-86-1, 1987.

15WP/PAP/seri

FUTURE OPERATION OF EXPERIMENTAL TEST UNIT ON MSW

D. P. Chynoweth, J. F. K. Earle, and D. E. Jerger

Bioprocess Engineering Research Laboratory
Agricultural Engineering Department
Institute of Food and Agricultural Science
University of Florida
Gainesville, Florida 32611

ABSTRACT

Research in our laboratory is concerned with conversion of renewable resources to methane and other useful products. This work which has addressed conversion of community wastes, including sewage sludge, aquatic plants resulting from reclamation of domestic wastewater and municipal solid waste, has revolved around an experimental test facility located at Walt Disney World Resort Complex in Orlando, Florida. We are proposing continued operation of this facility for process scale-up and its modification for testing of a variety of reactor configurations with emphasis on high solids digestion. Current laboratory research on MSW is described which is emphasizing feed analysis; effects of solids concentration, inoculum-to-feed ratio, and temperature on digester performance; process development research with emphasis on thermophilic digestion in high solids plug flow and leach bed attached film reactors; and wet air oxidation for posttreatment of digester effluent. A multidisciplinary approach to evaluation of microbial community structure is described, including use of fluorescent antibodies, nucleic acid probes, lipid analyses, and probes for in situ real-time measurement of NADH(NADPH) for total bacterial activity and F_{420} for measurement of methanogens.

INTRODUCTION

Research in our laboratory is focused on biological processes for conversion of renewable resources to methane. Feedstocks under study include municipal solid waste, wood, and grasses. We interact with a multidisciplinary coordinated team of researchers to address salient fundamental, and applied aspects of this conversion process in a manner needed to reduce

conversion costs through increasing conversion yields, rates, and process stability and simplifying process design and operation.

The general approach to anaerobic digestion process development research employed in our laboratory is outlined in Figure 1. Feedstocks are routinely analyzed for solids, organic content, heating value, and macronutrients in order to conduct materials and energy balances across the digestion process, evaluate potential nutrient limitations, and determine feed variability.

Figure 1. APPROACH TO ANAEROBIC DIGESTION PROCESS DEVELOPMENT

CHEMICAL AND PHYSICAL FEED ANALYSIS

ANAEROBIC BIOGASIFICATION POTENTIAL

- FEED VARIATION
- PROCESS VARIABLE EVALUATION
- PRETREATMENT

BENCH-SCALE PROCESS DEVELOPMENT

- CONVENTIONAL CSTR
- UNCONVENTIONAL
- MODEL DEVELOPMENT
- AUTOMATED DATA ACQUISITION AND PROCESS CONTROL

PROCESS SCALE-UP

- EXPERIMENTAL TEST UNIT (ETU)
- DEMONSTRATION

MICROBIAL SUPPORT STUDIES

A major thrust of this work is to develop models that predict anaerobic biodegradability based on these analyses. The biochemical methane potential assay is widely employed in our laboratory to evaluate extent and rates of fermentability to methane. We have recently shown reasonable correlation between rate constants developed from this method and those observed in semicontinuously-fed digesters. This assay is widely used to determine feed variability and evaluate optima for process variables such as inoculum, solids, pH nutrients, and temperature. It is also valuable in assessing effectiveness of various pretreatment techniques. Our bench-scale studies always include studies baseline CSTR reactors to provide reference data. A variety of unconventional digesters may be employed depending upon the nature of the substrate; some of these are outlined in Figure 2. Anaerobic digestion

models are under development at various levels of detail with plans of integration with an overall systems model. A major long-term goal is to develop an automated data acquisition and process control system employing measurements of methane production, temperature, pH, volatile acids which provide data which feed into a model that drives a process control operation system.

Figure 2. REACTOR OPTIONS FOR BIOMASS CONVERSION

BIOMASS TYPE	EXAMPLE	REACTOR OPTIONS
SOLUBLE (<2% SOLIDS)	WATER HYACINTH JUICE	ANAEROBIC FILTER FLUIDIZED BED EXPANDED BED UPFLOW ANAEROBIC SLUDGE BLANKET
LOW SOLIDS (2 - 10% SOLIDS)	WHOLE WATER HYACINTH	CSTR WITH SOLIDS RECYCLE NON-MIXED VERTICAL FLOW REACTOR
HIGH SOLIDS (>10% SOLIDS)	HYACINTH SOLIDS	CSTR LEACH BED ATTACHED FILM REACTOR PLUG FLOW REACTOR

A reasonable systems and economics assessment is dependent upon scale-up data which are developed at an intermediate scale digester such as the Experimental Test Unit at Walt Disney World. In general this unit provides verification of laboratory observations and data for materials and energy balances needed for accurate scale-up and costing. Microbial support studies are fundamental in nature and provide information leading to improved designs, operating conditions, and methods for evaluating performance.

I have been involved in the Community Waste Research Project at Walt Disney World since 1980 as project manager in charge of conversion research. Although this work initially focused on water hyacinth and primary sludge blends, it has also evaluated ensiled sorghum and currently MSW/sludge blend. After my move to the University of Florida in 1985, management of the ETU operations remained at IGT under the direction of Richard Biljetina and the University of Florida continued to be involved in experimental planning and provided of one of the ETU operators. Laboratory research was initiated at the University of Florida with focus on feedstock analysis, advanced digester development, and coordination of microbiological studies to evaluate community structure within

the reactor. Under the mutual agreement of the program participants the University of Florida will assume responsibility of management of ETU operation beginning January 1988. In the following paper I would like to discuss future operations of the experimental test unit and an overview of the status of our laboratory research.

EXPERIMENTAL TEST UNIT RESEARCH PLAN

Intermediate scale testing of specific feedstocks and digester concepts is necessary to obtain a valid data base for a systems and economic analysis and as a basis for further scaleup and commercialization. Specifically the objectives of scaleup in a system such as the experimental test unit are as follows: 1) verification of laboratory observations; 2) testing of selected feed processing and reactor configurations, 3) field scale evaluation of physical; chemical, and biological unit operations in an integrated manner; 4) determination of analytical and process control requirements; 5) compilation of preliminary information on maintenance and operating problems, equipment safety and scaleup effects; and 6) development of a valid data base for a systems and economic analysis. Richard Biljetina has just described the experimental test unit conversion facility at Walt Disney World. We are proposing the continued operation of this unit on municipal solid waste, future modification to include additional reactor options; and use by SERI, GRI, and other funding agencies as a scaleup test facility for anaerobic digestion of various feedstocks of interest. This unit has numerous features which make it attractive as a scaleup test facility for GRI, DOE and other funding organizations. It is located in a major city that is centrally located and easily accessible by air traffic. Orlando is an attractive area for holding scientific meetings and other project meetings. Because of this and the high amount of traffic at Disney World, the unit and associated projects would be frequently visited and highly visible. The unit is designed for a high degree of flexibility and can be easily modified to accommodate a variety of anaerobic digestion reactor options. It is an advanced design and capable of generating good balances across the system. There are on-site laboratory and office trailers which provide facilities for analyses as well as desk space for visiting researchers. I would like to stress the potential for the ETU to be converted to a variety of reactor options. Although the existing digester is designed for low solids feeds (up to 7% suspended solids) the facility currently has several other vessels which provide for easy modification to operate various other reactor options including CSTR, leach bed attached film, and attached film reactors of various types. Current plans include modification of one of the vessels to accommodate high solids feeding planned the next series of tests for MSW.

Representative performance data for several reactor designs for anaerobic digestion of refuse derived fuel (RDF) are shown in Table 1. We plan to use these data as a basis for selecting the next operating conditions for the experimental test unit. Although some differences in these data could be attributed to differences in RDF composition, the leading contenders on the basis of methane yield and methane production rate are DRANCO and VALORGA high solids digesters. Both designs receive RDF at solids concentrations over 40% and operate at internal solids concentrations in the 20-30% range. These reactors however need verification because detailed performance and operating data are proprietary and a fundamental laboratory data base for these fermentations is lacking. The leach bed attached film reactor design may have its own advantages because of the low cost associated with a landfill type hydrolysis phase and the fact that this reactor design facilitates production of enriched gas with over 90% methane. Based on these data we propose to simulate the DRANCO process in the ETU which will require modifications for high solids feeding and effluent recycle and removal. We are currently obtaining a laboratory data base to help focus the operating conditions for the first experimental run.

The proposed test plan for 1988 ETU operations is presented in Figure 3. Low solids experiments in progress will be completed. Feed handling tests will be conducted to determine the best method for ETU modification. The ETU will be modified so it can receive solids concentrations up to 50% or more. The unit will be modified during the months of January-March, shaken down in April, and tests begun in May 1988. During the first test, the unit will be operated under the conditions outlined for the DRANCO process, i.e., a temperature of 50°C, loading of 1 lb VS/Ft^3 day^{-1} and retention time of 20 days. Future operations will be planned in collaboration with GRI and SERI program participants.

STATUS OF LABORATORY SCALE RESEARCH

Three major goals of anaerobic digestion represent the emphasis of current research in our laboratory are to: 1) maximize high solids feeding and operation 2) improve the process reliability, and 3) upgrade the methane content of the gas during conversion. High solids operation has the advantages of minimizing feed and reactor heating requirements, reducing reactor size, increasing the temperature of operation and associated kinetics without significant heat losses, and reducing the amount of water handling requirements throughout the process. Digesters continue to be notoriously unreliable, in particular during startup, change in operating conditions, and in response to toxic feed components and over feeding. We have therefore dedicated a part of our work toward automating data acquisition and process control and understanding the

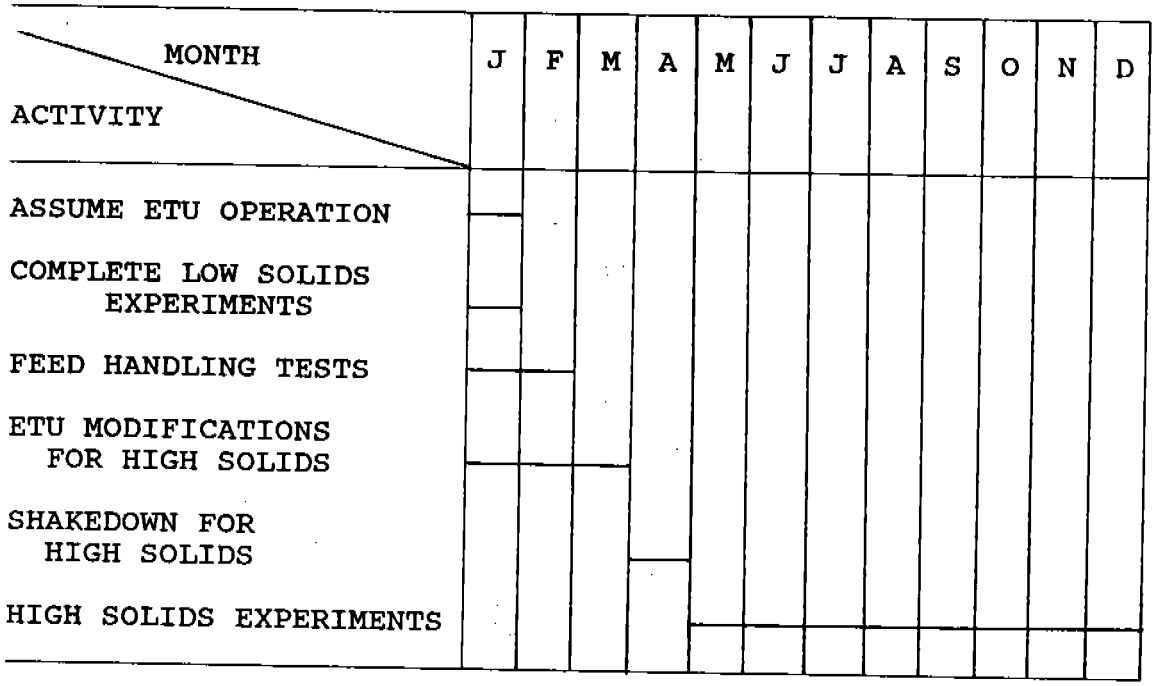
Table 1. REACTOR PERFORMANCE COMPARISON FOR ANAEROBIC DIGESTION OF MSW (RDF)

Reactor	REFCOM CSTR	Gaddy CSTR	IGT SOLCON	DRANCO	VALORGA Mixed	Ghosh LBAFR*	Nyns LBAFR*
Temp., C	60	30	35	50	37	35	35
Loading, lb VS/ft^3-day	0.19-0.6	0.22	0.1	1.0	0.94	0.07	0.48
Retention Time, d	6-27	80	27	20	15	90	15
Methane Yield, SCF/lb VS added	2.1-4.8	4.5	4.4	4.4	3.2	3.3	3.5
Methane Rate, V/V-day	0.91-1.30	0.99	0.44	4.4	3.0	0.24	1.7

*Leach bed attached film reactor

fundamentals of mechanisms giving rise to imbalances in the fermentation. The last goal of achieving high methane content is pertinent to producing pipeline quality gas (over 90% methane). This may be achieved through various reactor designs such as phase separation and gas stripping of a recycled leaching.

Figure 3. ETU WORK PLAN FOR 1988



The current research focus in our laboratory is outlined in Figure 4. We are seeking a method to distinguish the nonbiodegradable organics from the biodegradable organics by taking advantage of the fact that plastics and lignin are significantly less reactive chemically than other organic components. The biochemical methane potential assay is continuously used to evaluate new feeds and pretreatment techniques. We hope to develop front-end separation techniques using aqueous pulping, a technique unsuitable for thermal conversion processes. Our research on process variable optimization is focusing on solids concentration, inoculum-to-feed ratio, and temperature. Based on European data, it appears that a balanced anaerobic fermentation can occur at high solids concentrations (40-50%) typical of those associated with composting. Associated with high solids operation will be the requirement to provide the optimum inoculum-to-feed ratio and quality of inoculum to prevent imbalance from occurring. Because the heat of reaction becomes more significant at high

solids concentrations, high temperature digestion will be emphasized.

Figure 4. EMPHASIS OF MSW RESEARCH AT THE UNIVERSITY OF FLORIDA

FEED COMPONENT ANALYSIS

BIOCHEMICAL METHANE POTENTIAL ASSAYS

PROCESS VARIABLE OPTIMIZATION

- SOLIDS
- INOCULUM/FEED RATIO
- TEMPERATURE

REACTOR DESIGN

- PLUG FLOW
- LBAFR

PROCESS EVALUATION

PROCESS CONTROL

EFFLUENT MANAGEMENT

Two reactor designs are currently receiving emphasis. The plugflow reactor design similar to that of DRANCO and the leach bed attached film reactor (LBAFR). With the LBAFR we are emphasizing increasing the kinetics of the hydrolysis liquefaction reactions in the leach bed and prevention of methane from spilling over from the methane phase into the leach bed. Our efforts to evaluate community structure are continuing through collaboration with Macarios (florescent antibodies to evaluate methanogens) David White (lipid profiles), and Aldrich (microscopy to look at changes in particulate structure and associations of microorganisms with particulates). We are also evaluating the BioChem Technology probes for measurement of reduced coenzyme (total microbial activity) and F_{420} (activity of methanogens).

Research on automated data acquisition and process and control is emphasizing measurement of gas production, temperature, and pH with future plans for methane content, volatile acids, and pH. We hope that the coenzyme probes can be added to this repertoire of automated data acquisition. Process control will be effected through use of digester models and or expert systems models. A student in Chemical Engineering is evaluating a novel method of process control referred to a adaptive modelling where methane production is

measured and optimized in response to temperature change as a control variable.

The final area of emphasis in our laboratory is effluent management. In addition to routine effluent characterization which includes proximate and ultimate analysis as well as the biochemical methane potential, we are evaluating wet air oxidation as a method for post-treating digester effluent for potential recycle into the digestion process.

Some of the indicated research is not focusing specifically on MSW as it is generic to digestion of high solids feedstocks and to the overall anaerobic digestion process. We hope that this work will lead to new digester designs and optimized operating conditions as well as effective methods for process evaluation and control.

This research involves numerous laboratories at the University in Florida and a few at other institutions. We hope to encourage interactions with other researchers involved in the SERI program and utilize the experimental test unit at the Walt Disney site as a focal point for scaleup of reactor designs and testing the various required equipment for feedstock preparation and effluent management.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Gas Research Institute, Southern California Edison, and U. S. Department of Energy for current support of this project. We are grateful to Walt Disney Imagineering and associated industries for provision of the site for the ETU and their role in project management.

METHANE FROM COMMUNITY WASTE SYSTEMS ARCHITECTURE AND ENGINEERING SUPPORT

Robert Legrand
Biomass Energy Specialist
Carlos S.Warren, P.E., Ph.D.
Manager, Advanced Energy Division
Reynolds, Smith and Hills, A-E-P, Inc.
P. O. Box 4850 - Jacksonville, FL 32201; 904/739-2000

ABSTRACT

As part of the "Methane from Community Waste Systems" project, the nationwide potential of anaerobic digestion was assessed, focusing on the concept of central community waste-to-energy facilities. Municipal solid waste (MSW) has a nationwide potential of 1.2 to 1.6 quad per year (1 quad = 10E15 Btu = 1.055 EJ); sewage and related solids would add about five percent to this potential. Biodegradable industrial waste is a limited and largely rural resource; the availability of crop and logging residues as well as manures is severely limited for an urban conversion facility. Energy crops have by far the largest potential of all biogas resources. The availability of these different resources to major cities was assessed, using the concept of a surrounding rural hinterland. This analysis was performed on metropolitan areas of three different sizes and the percent of urban energy demand that could be satisfied by the community waste facility was quantified.

Since most of the feedstock to a community waste facility will be MSW, an economic comparison was performed of anaerobic digestion of trash versus mass burning, the most likely competing technology. Three possible uses for the biogas were considered, as well as three possible disposal strategies for the digested effluent. In most cases the break-even tipping fee for anaerobic digestion startss out lower than mass burning and declines, whereas the mass burn tipping fee increases over time.

A spreadsheet mathematical model of a community waste facility is being designed. It includes modules for MSW preprocessing, sewage treatment with water hyacinths, feed input, kinetics calculation, low solids anaerobic digestion, high solids anaerobic digestion, gas processing, digested residue processing, energy balance and levelized gas cost calculation. It also generates flowsheets with mass and energy balances for the main processes. Various residue processing options are considered, including landfilling, composting, incineration (without power recovery), combustion with power recovery, gasification, and wet oxidation.

METHANE FROM COMMUNITY WASTE SYSTEMS ARCHITECTURE AND ENGINEERING SUPPORT

INTRODUCTION

The objective of the "Methane from Community Waste Systems" project sponsored by the Gas Research Institute (GRI) and Walt Disney Imagineering (WDI) is the development of non energy-intensive waste treatment technology and the net production of energy (pipeline quality methane) from waste resources through anaerobic digestion.

This work has been proceeding at the Community Waste Research Facility on the grounds of Walt Disney World near Orlando, FL, for several years. Other participants include the Institute of Gas Technology (IGT) and the Bioprocess Engineering Research Laboratory (BERL). Secondary sewage treatment with water hyacinth ponds was developed (in contrast to commonly used tertiary treatment or polishing). A new type of anaerobic digester, the Non-Mixed Vertical Flow Reactor (NMVFR) was developed to biogasify the primary sewage sludge and harvested water hyacinths. The same reactor was used to convert sorghum to biogas and presently the organic fraction of municipal solid waste (MSW). The firm of Reynolds, Smith and Hills was contracted in 1986 to provide engineering services including an assessment of the nationwide potential of anaerobic digestion focusing on the concept of a central community waste/energy facility, a mathematical model of such a plant, site-specific studies, economic analyses and prospect assessment for a demonstration facility.

APPROACH

The size of each biomass or waste resource was estimated from data in the scientific literature and from government statistics; availabilities were estimated in part using geographical modeling. A mathematical model of the community waste/energy facility is constructed calculating mass balances, energy balances and a gas cost. Lifecycle cash flow analysis was used to compare different waste conversion options.

RESULTS

1.0 Biogas Potential of the U.S.

The potential supply of feedstocks available for biogasification in a community waste/energy facility was analyzed; urban and nearby rural waste and biomass were considered. Municipal solid waste (MSW) was studied first. For 1990, 5.3 to 7.0 pounds MSW (wet)per capita per day (2.4 to 3.2 kg/cap. day) is projected, resulting in 240 to 320 million tons (wet) (220 to 290 million metric tons) per year nationwide (see Figure 1). Extrapolating from experience with MSW biogasification, the corresponding nationwide methane potential from MSW is 1.2 to 1.6 quadrillion Btu (10^{15} Btu, quad) (1.3 to 1.7 EJ) per year. Sewage treatment at this facility would yield primary sludge and aquatic plant biomass/secondary sludge resulting in an additional nationwide biogas potential of 0.076 quad/year (0.08 EJ/yr). Tertiary sewage treatment with plants has a potential of 0.75 quad/year (0.79 EJ/yr) of biogas.

Biodegradable industrial waste overall could be used to generate 0.4 quad/year (0.4 EJ/yr). It is mostly lumbermill and sugar industry waste and is primarily generated in rural areas.

Crop residues remaining in the field after harvest are potentially a very large resource (400 million dry tons per year) that could generate 4.1 quad/year (4.3 EJ/yr). However, its availability is severely constrained by its dispersed nature, soil erosion concerns, and limitations in manpower and equipment. The practicality of using field crop residues as a biogasification feedstock can be enhanced by breeding crops so as to increase the tonnage of residue biomass produced per acre, without affecting the production of agricultural commodity. Of the logging residues, about one half are hardwood residues suitable for anaerobic digestion, with a potential of 0.3 quad/year; they are subject to constraints similar to the field crop residues.

Manure from confined animals could contribute 0.35 quad/year (0.37 EJ/yr); it is an excellent substrate from anaerobic digestion but not economically transportable because of its usually high moisture content. Energy crops are high yielding plants that convert well to biogas and are grown specifically for that purpose, yielding a gross income per acre competitive with conventional food and fiber crops. Using the area of cropland typically set aside in the U.S. to relieve agricultural overproduction, these energy crops could contribute 5.5 quad/year (5.8 EJ/yr) as methane; twice as much land can be made available, which would double this methane potential.

Thirty-mile wide belts of rural hinterland were drawn around major metropolitan areas; the rural biomass and waste generated within these hinterlands were assumed to be available to an urban conversion facility and was quantified for the continental U.S. This analysis was performed at three levels: all metropolitan areas down to 500,000, 200,000, and 100,000 inhabitants. Urban feedstocks were also included, based on nationwide figures, prorated to the number of people included in each category. The biomass and waste resource of metropolitan areas of at least 100,000 inhabitants could satisfy 43% of their gas demand. One third is urban waste and biomass (very available), 40% is rural resource available in high density central locations (less available), and one quarter is dispersed rural resource (largely unavailable).

2.0 Economics of MSW Biogasification

A plant converting MSW to gas would first incorporate a section where incoming trash is shredded and sorted, yielding a purified digester feed that is homogenized, enriched in biodegradable organics and devoid of large pieces or plastic stringers. This feed is similar to Refuse-Derived Fuel(RDF); it is then anaerobically digested and the digester effluent is dewatered. The resulting hot filtrate is recycled back to the digester to conserve heat, moisture, nutrients, alkalinity and active microbiota.

The economics of MSW mass burning, the leading trash disposal technology after landfilling, were compared with those of anaerobic digestion using a life cycle economic analysis. Energy prices, recyclables values and a comprehensive set of financial and economic assumptions are inputs.

Three possible uses for the biogas were considered:

- Direct sale to a nearby industrial/utility customer using a dedicated pipeline (the most economical option, but dependent on a long term contract).

- Combustion in a combined cycle gas turbine on the premises, with power sale to the grid, yielding 25 to 58% more power per ton of trash because of the higher efficiencies involved (economics dependent on high electricity resale rates).

- Upgrade to pipeline quality with sale of the resulting Synthetic Natural Gas (SNG) at citygate rates.

Three possible strategies for disposing of the digested and dewatered solid residue (filtercake) are also assessed:

- Combustion with power generation, yielding substantial excess power for resale to the grid.

- Combustion without power generation, requiring power purchase but avoiding the considerable investment of power generation.

- Landfilling without combustion, minimizing plant costs but maximizing residue disposal. Environmentally, this is still an improvement over landfilling of raw MSW, since the residue is much reduced in mass and bulk, is organically stabilized and heavy metals have been largely removed.

Two sizes of operation are included: 500 and 1000 tons per day (TPD). Typical capital and financing costs are illustrated in Table 1; note that the cogeneration plant includes full state-of-the-art air pollution controls.

Operating expenses include labor, operation and maintenance, residue/ash landfilling, and debt service. Revenue elements are the interest on the reserve fund, the sale of electricity/gas and recyclables, and the tipping fee. The latter is calculated so as to result in a zero cashflow and will be called the breakeven tipping fee.

The evolution of the breakeven tipping fee over time for mass burning versus anaerobic digestion is illustrated in Figures 2, 3, and 4. In Figure 2, a capacity of 500 tpd is assumed, biogas is cleaned up to SNG and three disposal options for the dewatered residue are considered. In Figure 3, for the same capacity and assuming the dewatered residue is burned with power generation, three uses for the gas are considered. Two scales of operation are included in Figure 4; it can be seen that economics of scale affect anaerobic digestion more than mass burning. This research is ongoing and the data are preliminary.

Many combinations of inputs have been tried, but the trend is generally the same: the tipping fee for anaerobic digestion starts out lower than that for mass burning and declines over time, whereas the mass burning tipping fee increases, in current dollars.

Table 1: RefCoM Plant Capital Costs and Bond Size (500 tpd, 7 days/week residue combustion and power generation, biogas to SNG)

Note: These costs can vary depending on contract agreements and site conditions

Front-end MSW feed processing	$ 9,350,000	(24.2%)
Anaerobic Digestion (10 digesters) including dewatering	9,632,000	(25.0%)
Cogeneration plant for combustion of dewatered residue and front-end oversize material to generate steam and electricity	17,320,000	(44.9%)
Gas cleanup to pipeline quality, 400 psi	2,250,000	(5.8%)
Total capital cost	$38,552,000	(100.0%)
Minus construction fund interest @ 7%	- 3,444,000	
Construction cost	$35,106,000	
Capitalized Interest @ 9%	10,158,000	
Dept Service Reserve (1 year)	5,500,000	
Underwriting Discount (2.2% of bond)	1,188,000	
Cost of Issuance (2.0% of bond)	1,080,000	
System Performance Insurance (5% of bond)	3,240,000	
Less Interest Income:		
Capitalized Interest Fund @ 7%	- 1,472,000	
.Debt Service Reserve Fund @ 7%	- 792,000	
Total Bond Size	$54,004,000	

3.0 Mathematical Model of a Community Waste Biogasification Facility

Work continues on a mathematical model of a community waste biogasification facility; the overall model architecture is summarized in Figure 5. The model will calculate mass balances, energy balances and levelized gas cost of service. The conversion facility accepts MSW, sewage, industrial waste and any other appropriate feedstock. MSW must first be processed including shredding, magnetic separation, sorting, etc.; this is quantified in the MSW front-end module: the quantity and quality of raw trash is entered here and, among others, the amount of refined digester feed is calculated. Sewage treatment involves primary sedimentation and a water hyacinth pond system. The existing Black and Veatch water hyacinth model was streamlined and part of it is used here to generate primary sludge and plant biomass production. The kinetics module generates a first order reaction rate coefficient from standard laboratory biochemical methane potential (BMP) data. The generated feedstock information, plus data about any other feedstocks that may be considered, is entered into the "Feed Input Module" where the data are processed for further use in the conversion modules.

A distinction is made between "dry" and "wet" feedstocks. Dry feedstocks such as MSW, have too little moisture for efficient anaerobic digestion, whereas "wet" feeds (sludge, water hyacinths) contain an overabundance of water. The two are best biogasified in different systems, to optimize water management and residue processing. Consequently, two parallel conversion trains exist; the dry side features dewatering of digested effluent and recycle of filtrate. The wet side is expected to result in a residue that can be marketed as a compost. Since excess moisture exists on this side, it can supply liquid to the dry side if needed. In an urban context, the dry side will be fed mostly MSW and therefore its residue will generally not be marketable as a compost because it will tend to be esthetically unacceptable. Therefore, a variety of residue processing options will be available here:

1) Landfilling;

2) Marketing as compost requiring thorough source separation of MSW. Agricultural residues, which can constitute an important fraction of the feed to the conversion facility, could increase marketability .

2) Incineration without power recovery;

4) Burning in a solid fuel boiler with full power generation;

5) Thermochemical gasification with combustion of the produced gas in a gas boiler with power generation;

6) Wet oxidation of digester effluent; this is a low temperature wet oxidation geared towards the production of organic acids to be converted in a fixed film digester.

The proposed community waste facility generates synthetic natural gas at pipeline pressure, so a gas processing module is included. Information about energy usage and production in every step is processed in the energy summary module. Finally, capital costs, O&M, energy product income, etc., will be used in the cost summary module to generate a levelized gas cost of service.

REFERENCES

1. Alich, J.A.; Inman, R.E. and 8 CoAuthors. "Crop, Forestry and Manure Residue Inventory - Continental United States (8 Volumes)", Stanford Research Institute, June 1976.

2. Ashare, E.; Leuschner, A.P.; West, C.E.; and Langton, B. "Assessment of Secondary Residues - Final Report (1981)". Prepared under Subcontract No. NR-9-8175-1 for SERI by Dynatech R/D Company.

3. Black & Veatch, Engineers-Architects; Walt Disney Imagineering. "Cost of Service Price for a Hyacinth Treatment/Methane Production System". Prepared for GRI, August 1986.

4. Hayes, T.D.; Isaacson, H.R.; Chynoweth, D.P.; Reddy, K.R.; and Biljetina, R. "An Integrated Wastewater-Energy Production System". Presented at "Energy from Biomass and Wastes X", April 7-10, 1986, Washington, D.C.

5. Legrand, R.; Coxe, E.F.; and Gambon, R.F. "Compared Economics Anaerobic Digestion vs. Combustion - Topical Report". Reynolds, Smith and Hills, A-E-P Inc., March, 1987. Prepared for GRI.

6. Legrand, R. and Warren, C. S. "Methane from Communited-Derived Waste Systems; A&E Support - Annual Report, 1986". Reynolds, Smith and Hills, A-E-P, Inc., February, 1987. Prepared for the Gas Research Institute.

7. Lipinsky, E.S.; Jenkins, D.M.; Young, B.A.; and Sheppard, W.J. "Review of the Potential for Biomass Resources and Conversion Technology - Final Report". Prepared by Batelle Columbus Laboratories for Gas Research Institute, July, 1983.

8. Pfeffer, J.T. "Evaluation of the RefCOM Proof-of-Concept Experiment". Presented at Energy from Biomass and Wastes X, April 7-10, 1986, Washington D.C.

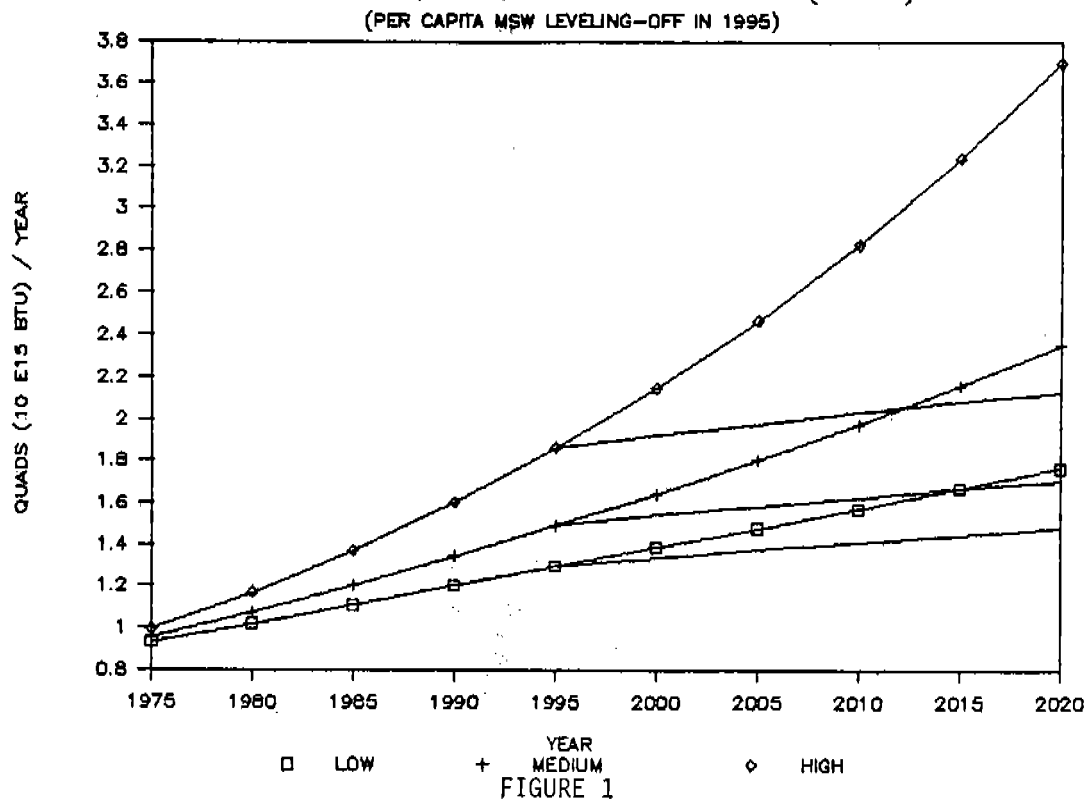


FIGURE 1

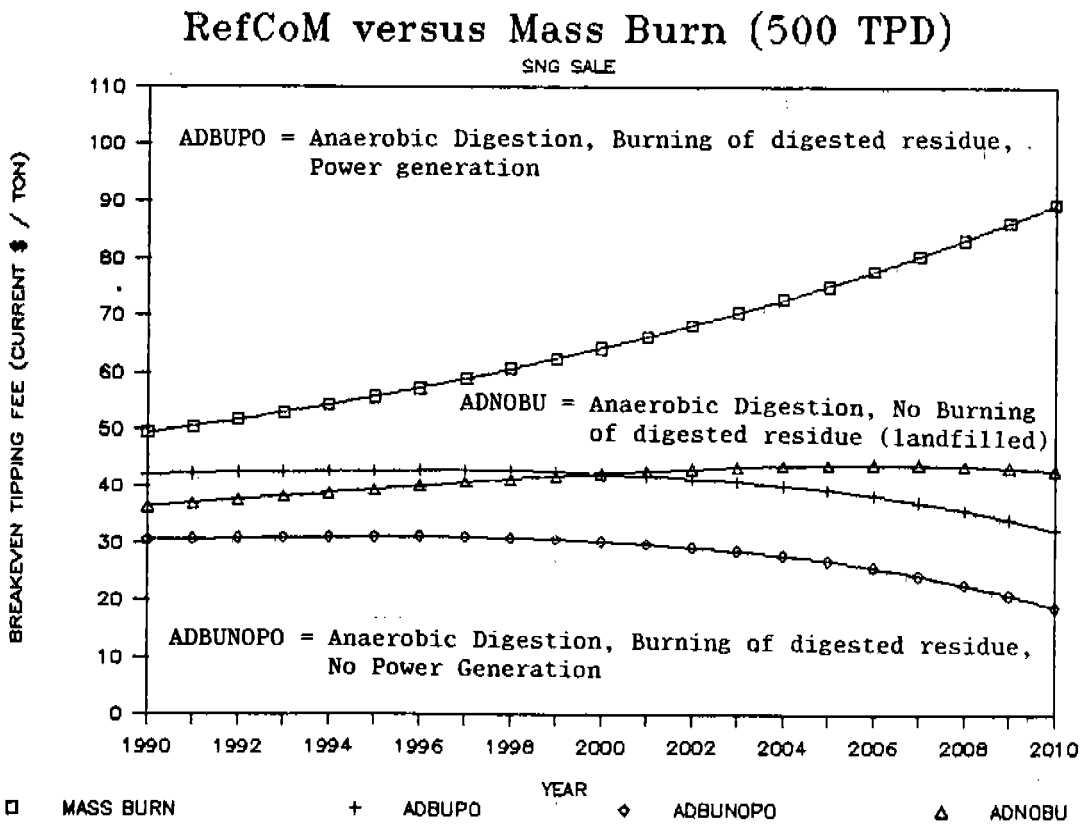


FIGURE 2

Mass Burn versus RefCoM : 500 TPD

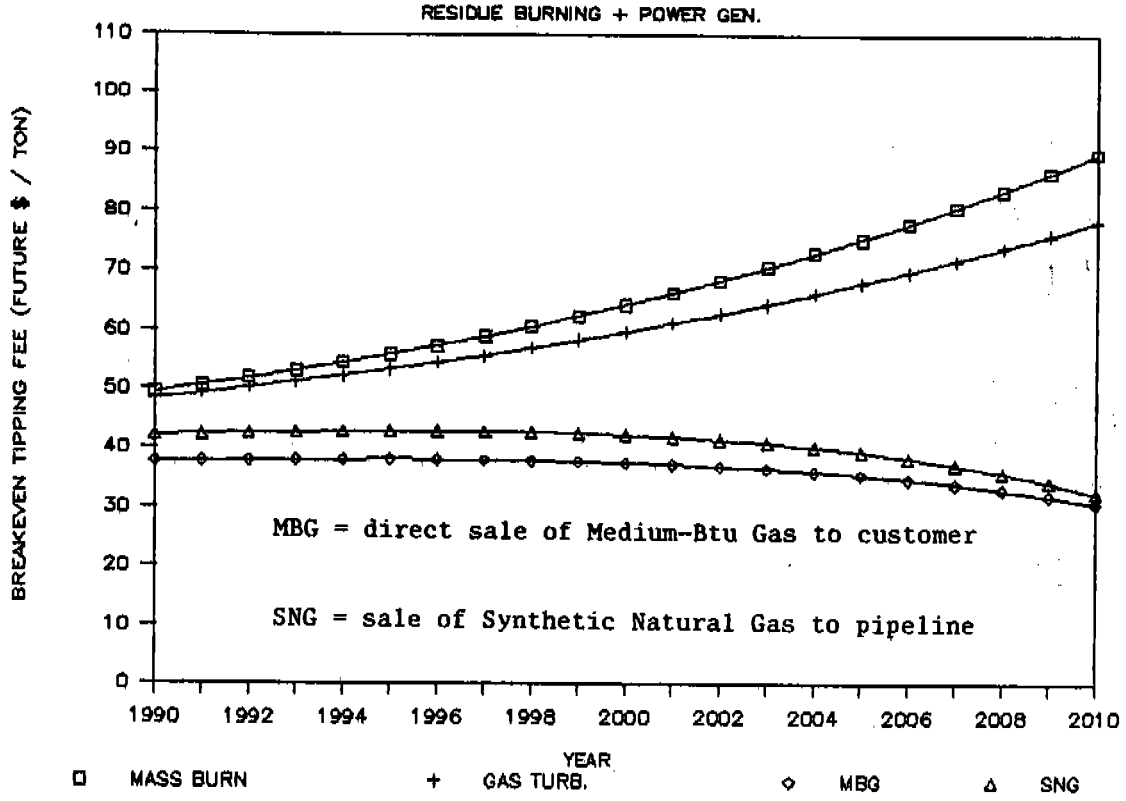


FIGURE 3

Mass Burn, RefCoM : 500 & 1000 TPD

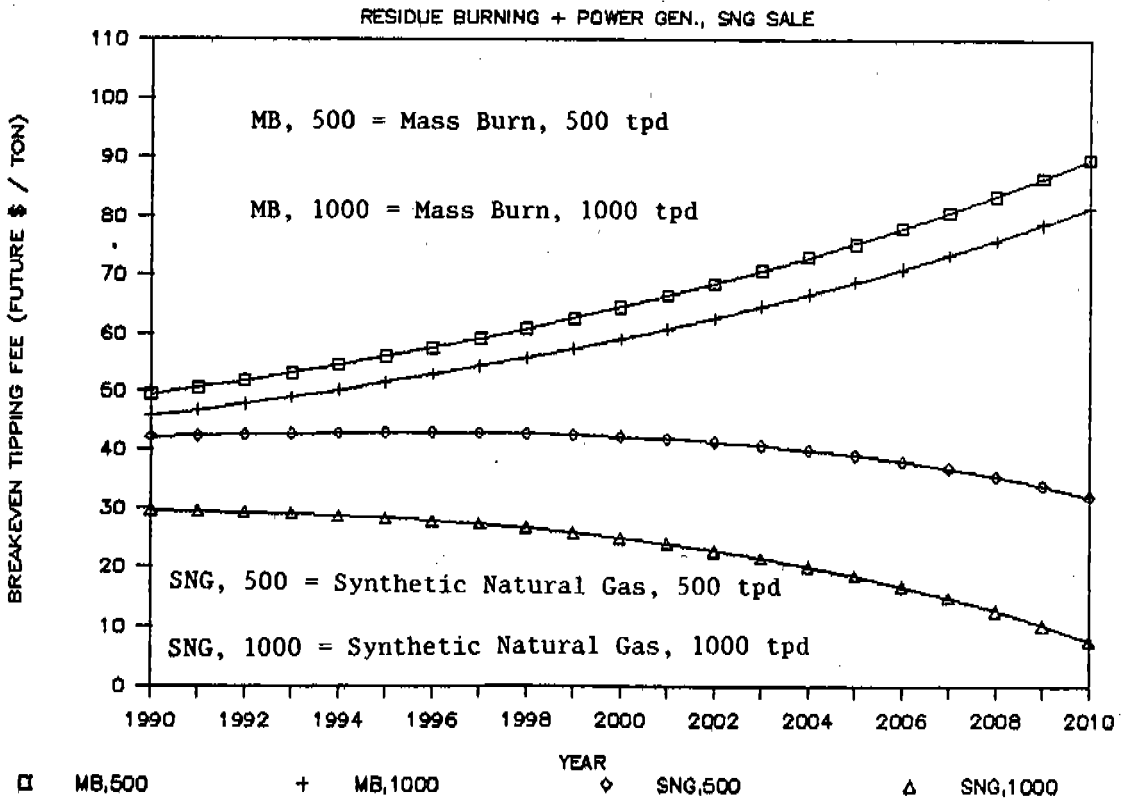


FIGURE 4

COMMUNITY WASTE FACILITY: MODEL ARCHITECTURE

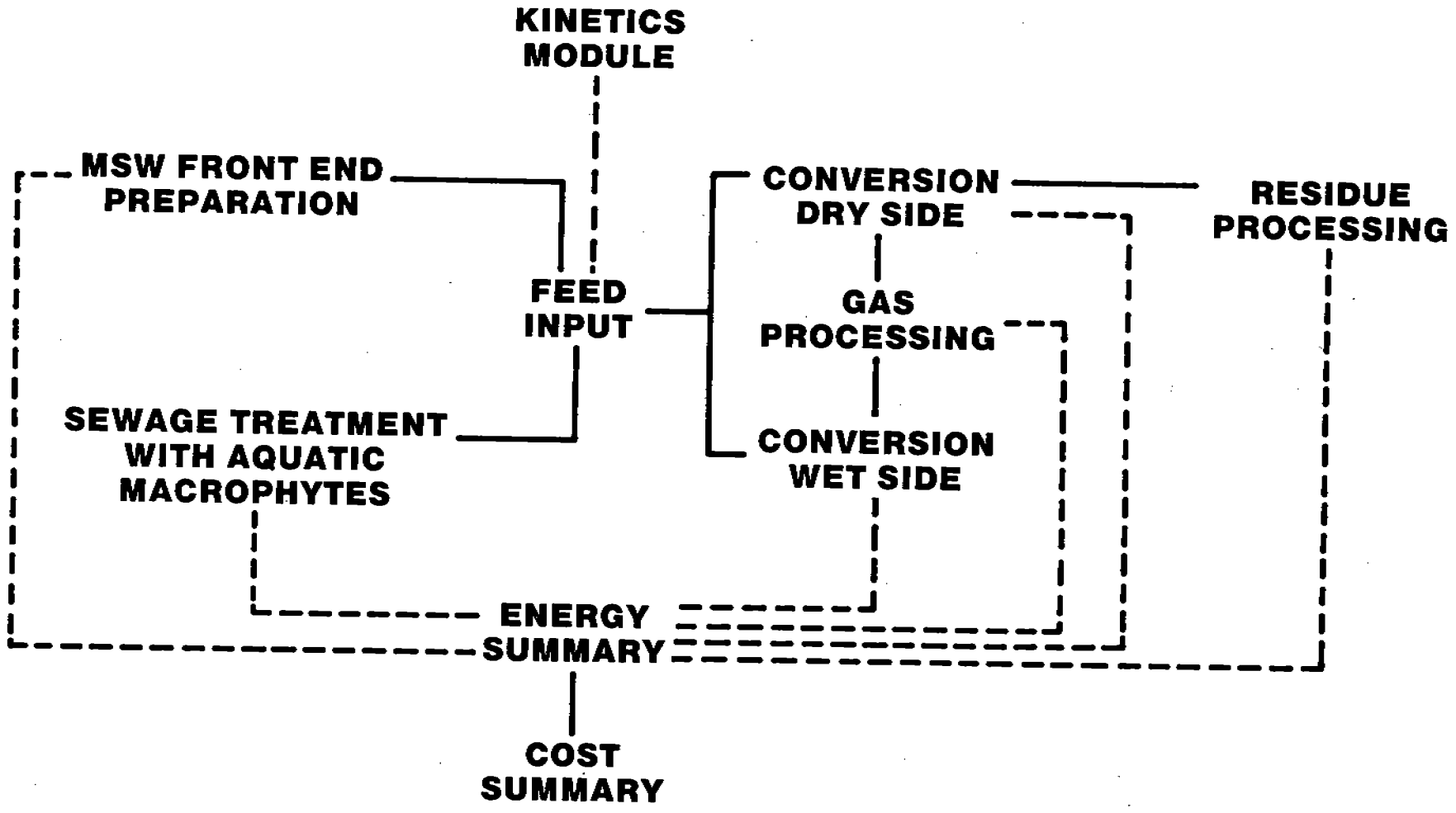
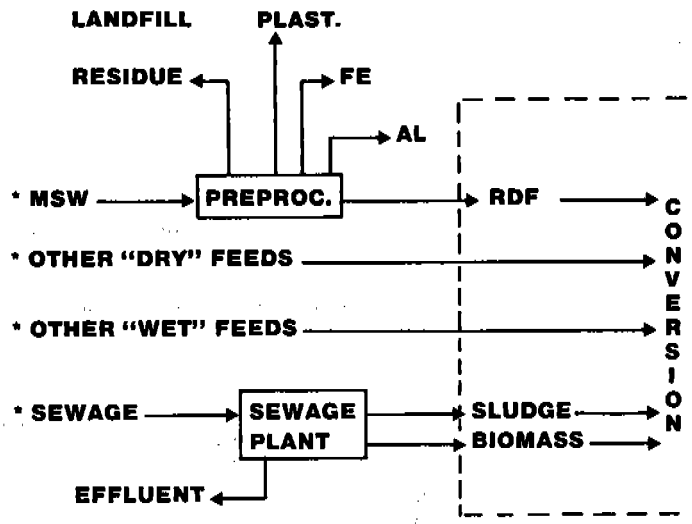
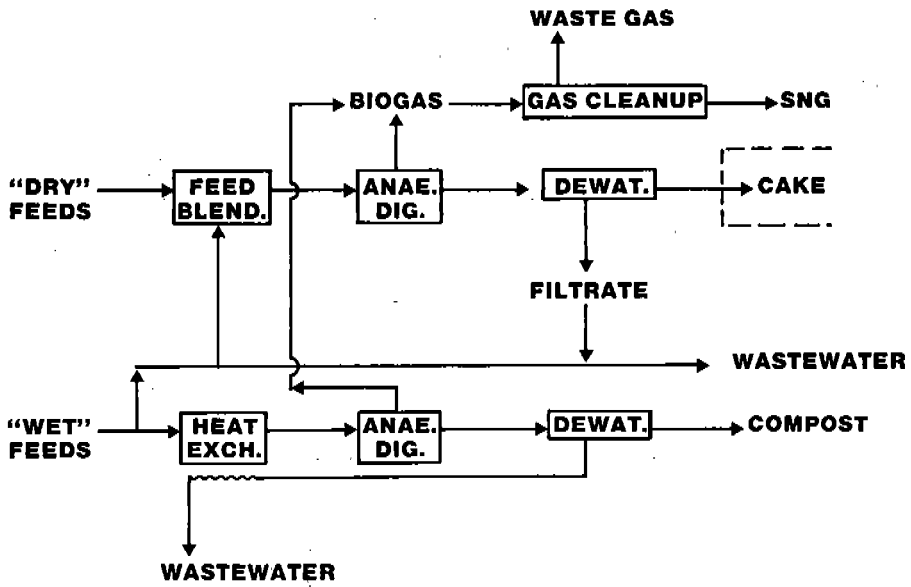
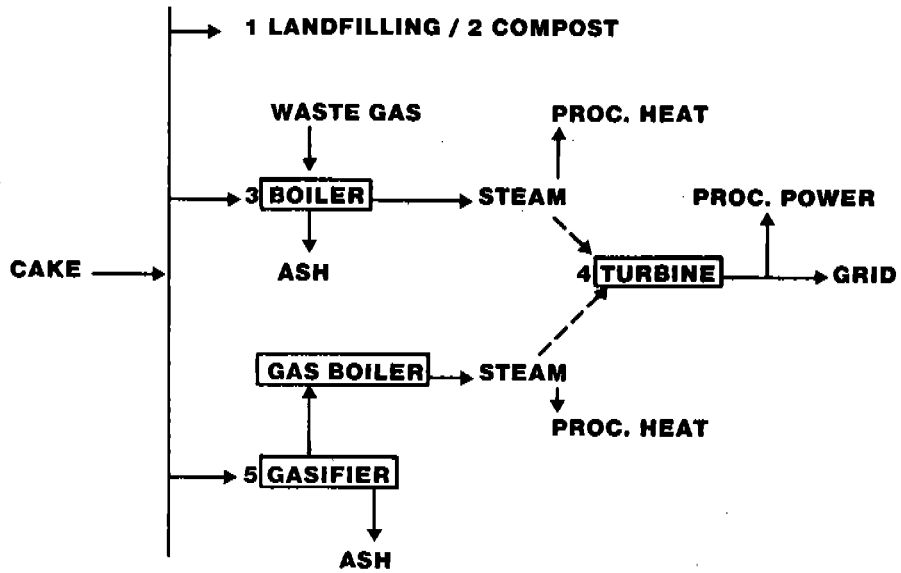
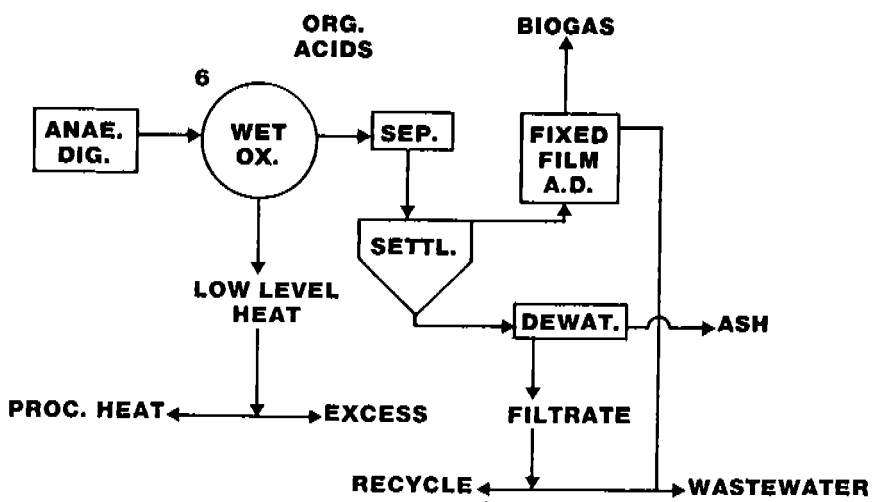


FIGURE 5


LANDFILL
PLAST.
RESIDUE
FE
AL
* MSW
PREPROC.
RDF
* OTHER "DRY" FEEDS
* OTHER "WET" FEEDS
* SEWAGE
SEWAGE PLANT
SLUDGE
BIOMASS
EFFLUENT
CONVERSION


WASTE GAS
BIOGAS
GAS CLEANUP
SNG
"DRY" FEEDS
FEED BLEND.
ANAE. DIG.
DEWAT.
CAKE
FILTRATE
WASTEWATER
"WET" FEEDS
HEAT EXCH.
ANAE. DIG.
DEWAT.
COMPOST
WASTEWATER


1 LANDFILLING / 2 COMPOST
WASTE GAS
PROC. HEAT
3 BOILER
STEAM
PROC. POWER
CAKE
ASH
4 TURBINE
GRID
GAS BOILER
STEAM
PROC. HEAT
5 GASIFIER
ASH


ORG. ACIDS
BIOGAS
6
ANAE. DIG.
WET OX.
SEP.
FIXED FILM A.D.
SETTL.
LOW LEVEL HEAT
DEWAT.
ASH
PROC. HEAT
EXCESS
FILTRATE
RECYCLE
WASTEWATER

Improved Reactor Concepts

PROCESSING HIGH SOLIDS CONCENTRATIONS OF MSW BY ANAEROBIC DIGESTION FOR METHANE PRODUCTION

E. C. Clausen, Professor
J. L. Gaddy, Professor and Head
Department of Chemical Engineering
University of Arkansas
Fayetteville, Arkansas 72701

ABSTRACT

Future research on anaerobic digestion must concentrate on reducing reactor volume and enhancing reaction kinetics to move this technology toward commercialization. The use of high solids concentrations for digestion of solid residues affords a potential means of reducing reactor volume by two-thirds. Solids concentrations are limited by conversion in the reactor, such that long retention times are necessary for higher solids concentrations. The use of cell recycle to enhance the kinetic coefficient and series operation of the reactors permit the use of lower retention times and higher solids concentrations.

This study has demonstrated the technical feasibility of concentrated solids digestion, cell recycle and series operation. Solids concentrations of 30 percent have been achieved and retention times as low as eight days have been attained without reduction of the reaction kinetics or conversion. Cell recycle and series operation have been demonstrated to permit lower retention times and higher solids concentrations. Further work should concentrate on defining the optimal system to minimize reactor volume.

PROCESSING HIGH SOLIDS CONCENTRATIONS OF MSW BY ANAEROBIC DIGESTION FOR METHANE PRODUCTION

INTRODUCTION

The United States generates 90 million tons of MSW (municipal solid waste) annually. At present, this material is collected and either burned or disposed of in a landfill. Landfills are reaching capacity and incineration raises public concern over hazardous emissions. A considerable fraction (70 percent) of MSW is carbohydrate: cellulosics (paper), lignocellulosic material (sawdust, wood, grass clippings, cardboard, etc.) and food waste. These materials are suitable substrates for the production of methane by anaerobic digestion, which can gasify up to 80 percent of the lignocellulosic waste to carbon dioxide and natural gas. Approximately 5 percent of the nation's natural gas requirement could be satisfied by the anaerobic digestion of MSW. The effluent by-product may be used as a nitrogen-rich soil amendment.

The capital costs are the single most important economic factor in the commercial application of MSW digestion. Sixty percent of the capital expenditures for a commercial-scale facility would be represented by the large reactors. The University of Arkansas is investigating methods to reduce the reactor volume requirement of anaerobic digestion. The specific research program is to evaluate the economic benefits of a number of high-solids loading schemes. These approaches include reducing reactor volume by using high-solids (30+ percent) with low retention times (20 days or less) and improving the reaction kinetics of the process by recycling effluent bacteria and by operating reactors in series. A brief review of the theoretical concepts of reactor volume reduction is presented, followed by a summary of the results achieved.

FACTORS AFFECTING REACTOR VOLUME

To determine the factors that influence the reactor volume, a summary of the reactor design procedure is presented. For a continuous flow reactor, the volume, V, is determined as the product of the volumetric flow through the reactor, F, and the retention time, θ:

$$V = F\theta \tag{1}$$

The flow rate is defined as the weight of the biomass introduced into the reactor, B, divided by the product of the solids fraction (by weight) in the biomass/water mixture, W_B, and the density of the mixture, d.

$$F = B/dW_B \tag{2}$$

The reaction rate is determined from a mass balance around the CSTR and the first-order kinetic relationship:

$$-r = \frac{C_i - C_o}{\theta} = kC_o \qquad (3)$$

where

$-r$ = reaction rate;
C_i = inlet substrate concentration;
C_o = outlet substrate concentration; and,
k = reaction rate constant.

Substituting Equations (2) and (3) into Equation (1) gives:

$$V = \frac{F(C_i - C_o)}{kC_o} = \frac{B}{dW_B} \frac{(C_i - C_o)}{kC_o} \qquad (4)$$

The conversion in the reactor, X, is related to biomass substrate concentration by the relation:

$$X = 1 - \frac{C_o}{C_i} \qquad (5)$$

Substitution of Equation (5) and rearrangement of Equation (4) gives:

$$V = \frac{B}{dW_B k} \left[\frac{X}{1-X}\right] \qquad (6)$$

The volume of methane, M, produced from a given quantity of biomass, is given by:

$$M = G\ B\ X = 6\ B\ X \qquad (7)$$

where G = gas conversion constant, depending upon the carbon content of the biomass and the methane concentration of the product gas. For a biomass that is 40 percent carbon and produces 50 percent methane, G = 360/12 x .4 x .5 = 6

Substituting Equation (7) into Equation (6) gives:

$$V = \frac{M}{6dW\ k} \frac{1}{1-X} \qquad (8)$$

Equation (8) can be used to analyze the parameters that influence the reactor volume and, hence, the process economics. For a required volume of methane production, M, the four factors that would lead to a reduction in the volume are:

a. an increase in the slurry density, d;
b. an increase in the biomass solids fraction in the reactor, W_B;
c. an increase in the reaction rate constant, k; or
d. a decrease in the biomass conversion, X.

Decreasing biomass conversion reduces reactor volume for a given methane production level and decreases the consumption of biomass. As the conversion is reduced, there is an increase in the quantity of effluent (incompletely reacted MSW solids, water, soluble organic and inorganic compounds) requiring disposal. Since one of the objectives of utilizing MSW is to reduce the volume of residue for disposal, merely reducing the conversion represents an impractical and unattractive, if not an expensive, approach to reducing reactor volume. However, reducing conversion would not be totally futile if innovative processes for effluent handing could be proven beneficial. These might include operation of reactors in series (effluent from reactor A is fed to reactor B rather than disposed of) or recovery of water from the effluent to decrease water consumption and disposal.

Another method for reducing the reactor volume is to increase the reaction rate constant. There are a number of possible ways to improve the reaction kinetics. One method, under investigation by others, is to pretreat the biomass to make it more readily digestible. Various pretreatment processes are under study, including the use of caustic, acid and high temperature (autocatalytic). An examination of the traditional Monod equation shows that the reaction rate, and, hence, the reactor volume, are dependent upon the microorganism concentration and the type of microorganisms. Other ways to improve the reaction rate constant would, therefore, involve increasing the microorganism concentration and improving the culture. Cell recycle, in which a portion of the microorganisms is returned to the reactor, is a method under examination in this project.

The reactor volume for a given amount of methane production varies in inverse proportion to changes in the density of the culture, according to Equation (8). However, the density inside the reactor cannot be increased beyond the point of fluidity, since water is more dense than solid MSW. The density can be decreased by adding MSW in concentrations greater than 10 percent, the maximum solids concentration for fluidity. When this occurs, slurry mixing, critical to gas production by promoting contact between MSW and the culture, is difficult and mass transfer and reaction kinetics are impaired. For consistently high gas production, fluidity must be maintained inside the reactor. For a water slurry of lignocellulosic material such as MSW, fluidity is not possible for mixtures exceeding about 10 percent (by weight) in water. It should be noted that higher solids concentrations (under study in some systems for inexpensive reactor designs) result in decreasing the density, which increases the reactor volume proportionately, according to Equation (8).

Perhaps the most promising method of decreasing reactor volume is to increase the biomass fraction, W_B, into the reactor, which results in a proportionate decrease in reactor volume. In the past, the upper limit of solids in the feed fraction has been considered to be 10 percent, such that the mixture could be pumped into the reactor. However, it is possible to feed more concentrated mixtures by introducing the solids and liquids separately or by utilizing other devices for feeding concentrated slurries. The concentration within the reactor must be maintained at 10 percent or less; therefore, conversion places an upper limit on the feed concentration.

POTENTIAL OF HIGH SOLIDS DIGESTION

The maximum allowable feed solids concentration is dependent upon the conversion of MSW in the reactor. The conversion in the reactor increases with increasing retention time, so that high retention times are necessarily required to achieve feed solids concentrations of 25-30 percent.

Table 1 presents the maximum allowable feed solids concentration as a function of conversion, assuming a 10 percent slurry concentration inside the reactor. The solids concentrations shown in the table can only be realized if the mass transfer between substrate and organisms inside the reactor allows conversions nearly independent of solids concentration. Thus, a good agitation system is essential to allow contact of substrate and organisms and to allow gas escape from the liquid surface.

Table 1

Maximum Allowable Solids Concentration
With Conversion

MSW Conversion	Maximum Feed Solids Concentration Weight Percent
20	12.5
30	14.3
40	16.7
50	20.0
60	25.0
70	33.3

If a feed solids concentration of 30 percent is possible to a mixed flow reactor without a decrease in conversion in comparison to a "standard" 10 percent feed, the reactor volume to an industrial facility can be decreased by 67 percent. This volume reduction translates to a proportional decrease in reactor capital costs. Marginal economics can thus be changed to favorable economics by incorporating this simple change.

EFFECT OF CELL RECYCLE ON MSW DIGESTION

The 3-liter stirred tank reactors used in this study of the anaerobic digestion of MSW contain a mixture of MSW, water and a complex mixture of microorganisms. Operated in continuous fashion at constant volume, effluent is removed at the same rate as fresh substrate is added. In a microbiological reactor, the effluent contains not only unreacted substrate, but also the biocatalyst. When operated at low retention times, the continuous loss of a proportionate amount of microorganisms in the effluent results in a net decrease in the cell concentration of the culture. A condition known as

"washout" occurs if reproductive multiplication of the microorganisms does not occur as fast as they are removed with the effluent. In anaerobic digestion, the slowest-growing variety of organisms, and therefore those most susceptible to "washout", are the methanogens, responsible for the conversion of acetic acid or CO_2 and H_2 to methane.

As a method to achieve lower retention times (prevent cell washout) and to increase conversion, cell recycle may be used in combination with high solids anaerobic digestion. Cell recycle is a technique of recovering cells from the effluent for recycle back to the feed. By utilizing cell recycle, less substrate is required for cell growth and, thus, more substrate is available for the production of methane. Also, less water is required for make-up since liquid is also recycled with cells. Washout is prevented (or, at least, slowed) since the cells are recovered instead of being discarded.

Cell recycle, as a technique for increasing conversion and preventing washout, is best applied at low retention times. The effects of cell recycle at an 80 day retention time, for example, would be minimal since the conversion in the reactor is already 70 percent (90 percent of theoretical). At a 10 day retention time, on the other hand, the conversion is only 31 percent, or 40 percent of theoretical. Therefore, a larger potential improvement can be achieved at low retention times with cell recycle.

SERIES OPERATION

Examination of Equation (3) shows that for MSW digestion, the reaction rate is proportional to the concentration of substrate in the reactor, C_o. For reactors designed for high conversion, C_o is low and, consequently, the reaction rate is slow. To achieve the same high conversion, two or more reactors could be arranged in series with effluent from the first as feed to the second, etc. The last reactor in the series has the same effluent concentration, C_o, and the same low reaction rate as a single reactor achieving the same conversion. However, preceding reactors have higher concentrations and the overall reactor rate is higher.

An infinite number of stirred-tank reactors (plug flow) would take maximum advantage of the concentration profile and achieve minimum reactor volume. The ratio of the volume of a plug flow reactor to the volume of a stirred-tank reactor, V_p/V_m, is shown in Equation (9):

$$\frac{V_p}{V_m} = \frac{(1-X_A)\ \ln\ (1-X_A)}{X_A} \qquad (9)$$

The ratio of V_p/V_m is illustrated in Table 2. Note that the ratio is smaller as the conversion increases. Therefore, in MSW digestion, where high conversions are desirable, series operation is most beneficial.

Table 2

Comparison of Reactor Volumes for Plug and Stirred-Tank Reactors

X_A	V_p/V_m
0.1	0.95
0.2	0.89
0.4	0.77
0.6	0.61
0.8	0.40
0.9	0.26

Plug flow reactors are suitable for gas-phase and liquid phase reactions. However, for slurries such as in the anaerobic digestion of MSW, plug flow reactors cannot be employed successfully since inoculation and solids removal would be impractical. However, operation of two or three reactors in series could be beneficial, especially at low retention times.

RESULTS

High Solids MSW Digestion

A summary of the results of high solids digestion, along with 10 percent solids digestion data, is shown in Table 3. As noted, the feed solids concentration ranged from 10 to 30 percent, with the retention time ranging from 10 to 100 days. These data represent the practical limits of digestion; that is, retention times greater than 100 days or less than 10 days are not appropriate, and solids concentrations above 30 percent are not possible while maintaining fluidity.

Based upon the high solids data of the experimental study and observations made during the study, the maximum MSW solids concentration fed to a reactor as a function of retention time can be obtained. The maximum solids concentration for fluidity inside the reactor has a practical limit of about 9 percent. The 10 percent level previously reported can only be sustained for short periods of time due to mass transfer limitations inside the reactor.

Combining these two results, Figure 1 presents the practical limit of MSW feed solids concentration that can be fed to a digester. As noted, a feed solids concentration of about 30 percent can be obtained at 80 or 100 day retention times. At a 10 day retention time, on the other hand, a maximum of about 13 percent is possible.

The benefit of high solids digestion would be diminished if the reaction kinetics are impaired. Figure 2 is a plot of the inverse conversion and inverse retention time from which the kinetics of high solids digestion can be obtained. The slope of the line in Figure 2 yields a rate constant of 0.068

Table 3

Anaerobic Digestion of MSW to Methane

Feed Solids Concentration (wt. percent)	Retention Time (days)	Gas Production (L/day)	Carbon Concentration (gmole/L) C_{in}	C_{out}	Reaction Rate (gmole/L·day)	Conversion (percent)
10	10	7.68	3.682	2.539	0.1143	31.0
10	10	7.54	3.682	2.560	0.1122	30.5
10	15	7.08	3.682	2.102	0.1053	42.9
10	15	6.91	3.682	2.140	0.1028	41.9
10	20	5.79	3.682	1.713	0.1862	46.8
10	30	4.41	3.682	1.713	0.0656	53.5
10	40	3.86	3.682	1.383	0.0581	63.1
10	40	3.90	3.682	1.359	0.0581	63.1
10	60	2.83	3.682	1.155	0.0421	68.6
12	10	10.29	4.418	2.887	0.1531	34.7
12	10	10.51	4.418	2.854	0.1564	35.4
15	20	5.89	5.523	3.779	0.0876	31.7
20	40	6.67	7.364	3.394	0.0993	53.9
20	60	5.43	7.364	2.520	0.0807	65.8
20	80	4.29	7.364	2.263	0.0638	69.3
20	100	3.44	7.364	2.252	0.0511	69.4
25	60	6.37	9.205	3.520	0.0948	61.8
25	80	5.45	9.205	2.712	0.0812	70.5
30	80	6.13	11.046	3.748	0.0912	66.1

$days^{-1}$, identical to the result obtained from the data for 10 percent solids digestion only. The ultimate conversion, calculated from the intercept of the figure, is within five percent of the 0.77 calculated from the ten percent solids digestion data. Therefore, the kinetics and ultimate conversion are not affected by utilization of high feed solids concentrations.

Cell Recycle

Retention time may be reduced and solids concentrations increased by the use of cell recycle. Table 2 summarizes the cell-recycle data obtained for the steady-state digestion of MSW to methane at four retention times and four different solids concentrations. Also included in the tables are steady-state data or predicted values for similar reactors operated without the benefit of cell recycle.

Retention times as low as 8 days have been proven feasible with cell recycle, when ordinarily this low retention time would result in washout. Thus, cell recycle is a technique that can be used to increase conversion, reduce cell washout, or increase solids concentrations to reactors. Solids loading to reactors may be increased by 25 percent at a 10 day retention time or 50 percent at 20 day retention time by employing recycle, which means that proportional decreases in total reactor volume are made possible by this technique.

Table 4

Effect of Recycle on Steady-State MSW Digestion

	Solids Retention Time	Recycle Rate (days)	Gas Production (L/day)	Carbon Concentration (gmole/L) C_{in}	Carbon Concentration (gmole/L) C_{out}	Reaction Rate (gmole/L·day)	Conversion (percent)
A.	20%	--	6.67	7.364	3.394	0.0993	53.9
	40 days	25	7.08	7.364	3.150	0.1054	57.2
B.	15%	--	5.89	5.523	3.779	0.0876	31.7
	20 days	50	9.79	5.523	2.609	0.1457	52.8
C.	12.3%	--(ND)	9.41	4.529	3.130	0.1398	30.9
	10 days	50	10.27	4.529	3.001	0.1528	33.7
D.	11.6%	--(ND)	9.58	4.271	3.131	0.1425	26.7
	8 days	50	10.13	4.271	3.065	0.1508	28.2

Series Operation

A summary of the results for series operation of CSTR's, with and without recycle, is shown in Table 3. Also included are predicted rates of gas production for single stirred-tank reactors of equal volume to the overall series reactor volume.

As the data indicate, significant improvement in reaction rates and conversion occur when reactors are operated in series, especially at lower retention times and higher solids concentrations. At a 45 day overall retention time, series experiment I yielded a 6.6 percent higher conversion than predicted without series operation. When the overall retention time was decreased to 20 days, the relative conversion improvement increased to 13.9 percent.

Table 5

Steady-State MSW Digestion by Series Operation of CSTR's, with and without Recycle

Series # Solids (percent)	Retention Time (days)	Gas Production (L/day)	Carbon Concentration (gmole/L) C_{in}	Carbon Concentration (gmole/L) C_{out}	Reaction Rate (gmole/L·day)	Conversion (percent)
I. CSTR's IN SERIES						
S-I, 10%	15 x 3	10.69	3.682	1.296	0.0530	64.8
9-Liter CSTR	45 (ND)	10.03	3.682	1.443	0.0498	60.8
S-III, 12%	10 x 2	15.25	4.418	2.149	0.1135	51.4
S-IV, 12%	10 x 2	15.31	4.418	2.140	0.1139	51.6
6-Liter CSTR	20 (ND)	13.42	4.418	2.421	0.0999	45.2
II. CSTR's IN SERIES WITH 50% CELL RECYCLE						
S-V, 11.6%	8 x 2	16.56	4.271	2.300	0.1232	46.1
6-Liter CSTR	16 (ND)	14.57	4.271	2.536	0.1084	40.6
S-VI, 12.3%	10 x 2	15.98	4.529	2.151	0.1189	52.5
6-Liter CSTR	20 (ND)	13.76	4.529	2.481	0.1024	45.2

(ND): Not demonstrated, values given are predicted from previous MSW digestion data

The addition of cell recycle to series operation improved reactor performance further. At a 20 day retention time, the conversion was 16.2 percent higher; at 16 days, the conversion was 13.5 percent higher, yielding the highest reaction rate achieved in series experiments.

CONCLUSIONS AND FUTURE WORK

MSW can be converted to methane in anaerobic digesters utilizing feed concentrations as high as 30 percent by weight without impairing the yield or reaction kinetics. Identical first order rate constants of .068 days obtained for 10, 20, 25, and 30 percent solids. The maximum feed solids concentration as a function of retention time has been determined for retention times ranging from 8 to 100 days.

The benefits of cell recycle on high solids digestion have been demonstrated in feasibility studies at retention times less than 40 days. Recycle experiments have been performed at retention times ranging from 8 to 40 days, with solids concentrations ranging from 11.6 to 20 percent. Cell recycle has been shown to be a technique for increasing the feed solids concentration to an anaerobic digestion, as well as a method to decrease the minimum retention time for a digestion. Feed solids concentrations as high as 12.3 percent by weight at a 10 day retention time and retention times as low as 8 days have been utilized in the presence of cell recycle.

The benefits of series operation have been demonstrated, comparing the performance of two or three MSW-digesting reactors in series with a single larger reactor of equal volume. Individual reactors in series have been operated at retention times ranging from 8 to 15 days. Overall conversions have been increased by 14 percent with series operation. The enhancement of series operation by cell recycle has also been demonstrated with increases in conversion of 16 percent over high solids digestion attained.

The technical feasibility of both cell recycle and series operation of high solids digesters has been demonstrated. Additional data need to be accumulated so that optimization studies can be performed. The optimal system will probably consist of a combination of high solids, recycle, series, retention time, and conversion that results in a minimum reactor volume. Studies need to be conducted to define these conditions for the optimal system.

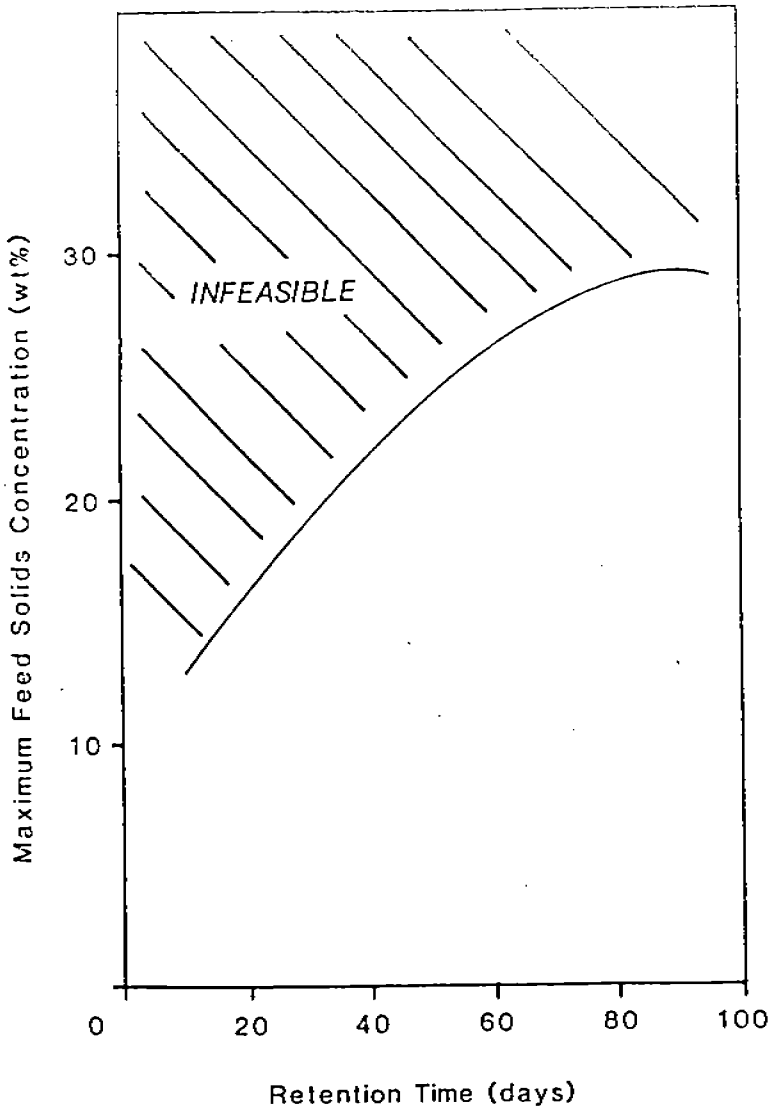


Figure 1. Maximum feed solids concentration at a given retention time.

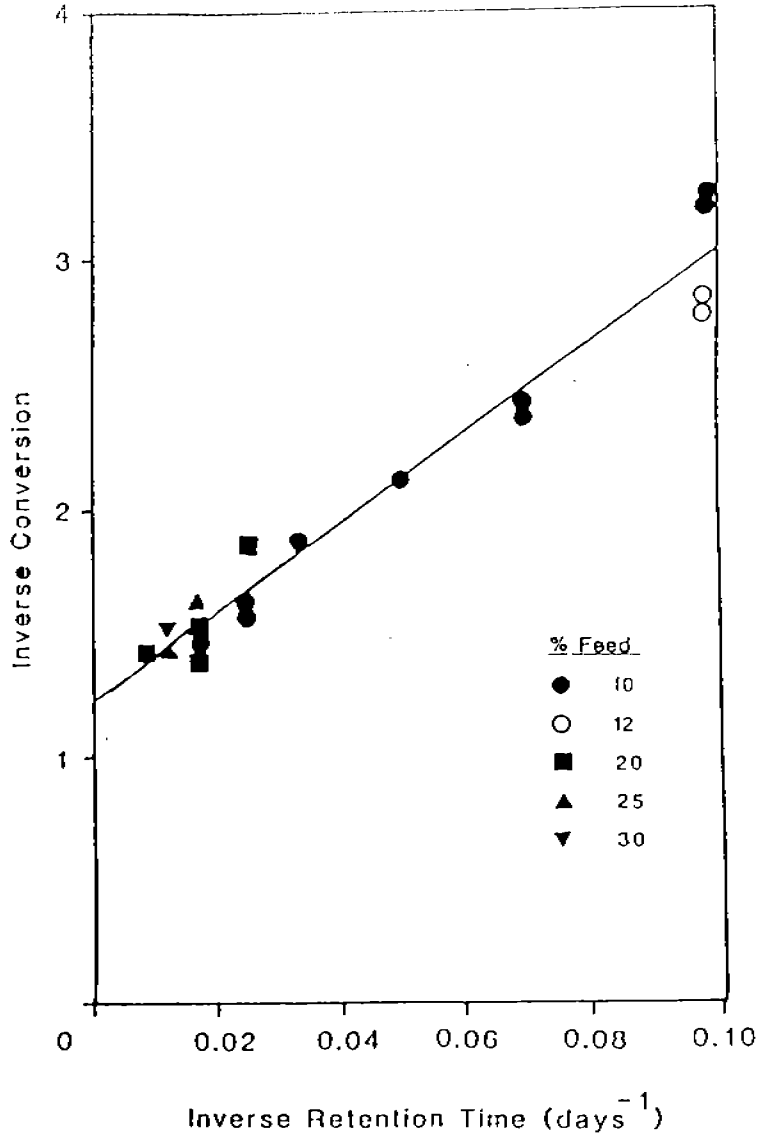


Figure 2. Conversion-retention time relationship for all MSW digestion data.

PROCESSING CELLULOSIC SOLIDS FOR METHANE PRODUCTION BY A COMBINED CHEMICAL AND BIOLOGICAL PROCESS

Gow-Jen Tsai and George T. Tsao
Laboratory of Renewable Resources Engineering
A. A. Potter Engineering Center
Purdue University
West Lafayette, IN 47907

ABSTRACT

Cellulosic solids are pretreated by calcium hydroxide to produce salts of volatile organic acids and other water-soluble substances. Pure cellulose, sawdust, and waste paper are used as model substances for the study of alkaline degradation. It was found that sawdust is more difficult to degrade than the other two substances. The cooking conditions for high conversion of model substance and high yield of organic acids are found to be 275°C to 300°C with the corresponding reaction time from 30 to 15 minutes. The cooking liquor can be readily fermented in an anaerobic fluidized-bed digester for methane production. The cooking liquor from different reaction conditions can all be digested by the methanogens. Higher than 90% of COD can be removed under the conditions of low organic loading rate (< 2.0 g COD/ℓ /day) and low hydraulic retention time (1.5-2.0 days).

PROCESSING CELLULOSIC SOLIDS FOR METHANE PRODUCTION BY A COMBINED CHEMICAL AND BIOLOGICAL PROCESS

INTRODUCTION

Anaerobic digestion of municipal solid wastes (MSW) to provide methane for energy is a well-known biological process. The product gas has moderate heating value and can be upgraded to very desirable pipeline gas by removing carbon dioxide. The supply of liquid fuels and chemicals from methane is also possible by catalytic routes [Haggin 1987; Haggin 1987]. However, the anaerobic digestion of lignocellulosic municipal solid wastes (MSW) is a very slow process which requires a long substrate retention time ranging from 20 to 100 days [Clausen and Gaddy 1984]. Also, the conversion is hardly complete; leaving, in many cases, as much as 50% of the organic materials unconverted. It was believed that the overall digestion process was controlled by the rate of degradation and solubilization of cellulosic materials in the MSW [Jeris and McCarty 1965].

An alternative route in processing such municipal solid wastes for methane production is to increase the degradation rate of cellulosic materials by pretreating them into more digestible substances which become the feed for the anaerobic digester. The pretreatment of cellulosics can be carried out by physical and chemical methods such as size reduction, alkali-cooking, steam explosion, freeze explosion, mild acid hydrolysis, solvent dissolution, and irradiation [Tsao 1984]. Among these, alkali treatment is the best known method of enhancement of bio-digestion of cellulosics [Colleran et al, 1982; Yang and Chang 1985]. This pretreatment converts cellulosics into water-soluble organic materials which can be directly digested by methanogens. Therefore, the combined process we proposed includes the alkali-cooking of cellulosics and the anaerobic digestion of cooking liquor.

This paper presents the experimental results of this combined chemical and biological process and discusses the technical feasibility of this alternative route. The overall process is schematically shown in Figure 1. Here, we only investigate two major portions of the process, i.e., alkali-cooking of cellulosics and anaerobic digestion. An anaerobic fluidized-bed digester has been employed to test the digestibility of the cooking liquor. This kind of digester retains the growth support media in suspension by drag forces exerted by the upflow of recycling liquid stream. The formation of biofilm on the carrier increases the ratio of solids retention time (SRT) to hydraulic retention time (HRT) which is desirable for process stability and minimal sludge production. Therefore, this combined chemical-biological process should have the merits of low substrate retention time and a high yield of methane.

EXPERIMENTAL

Alkali Pretreatment

The alkali pretreatment of cellulosics in MSW was carried out in micro-tubular, preparative-tubular, as well as pilot-scale tubular reactors.

The micro-tubular reactors were made of stainless steel tubings (316, 3/8" O.D.) with caps on both sides. These reactors have a capacity of 6 ml and they are used

essentially for kinetic studies. Pure cellulose (Avicel, PH 101), black oak sawdust, and waste paper (Fort Howard Paper Company) were used as model substances for the test. A certain amount of model compound was well-mixed with calcium hydroxide and then the mixture was transferred to the reactor. Distilled water was added to adjust the weight percentage of model compound in the reaction medium. The mixture in the reactor was well-mixed by using a vortex stirrer and the reactors were then placed on the shaker for one to two hours to allow the alkaline solution to be adsorbed by the model substance. Meanwhile, the sand bath was preheated to the reaction temperature. Lastly, these micro-tubular reactors were immersed into the sand bath with a heat-up period of less than one minute.

The micro-tubular reactors were withdrawn from the sand bath one at a time by a predetermined time sequence. The reactor was quickly transferred to a pail of tap water to quench the reaction and was opened to collect the cooking liquor as well as the residue after the temperature of the reactor decreased to the room temperature. Filtration was employed to separate the residue from the cooking solution. The residue was then washed with distilled water which diluted the cooking solution to a total volume of 35-50 ml. The residue was further washed with 1N HCl to remove unreacted calcium hydroxide; it was then washed with distilled water, dried, and weighed. The overall conversion can be determined by the weight difference of model compound before and after the reaction.

Five preparative-scale tubular reactors were also fabricated to provide feed for the anaerobic digester as well as to provide information for process scale-up. They were also made of stainless steel tubing (304, 1") with a capacity of 100 ml. The experimental procedure is the same as that mentioned above for micro-tubular reactors.

The pilot-scale tubular reactor was locally designed and fabricated and was also employed to provide enough cooking liquor for the digester. The maximum capacity of this reactor is ten liters. The reactor consists of three sections: feeding zone, heating zone, and cooling zone. A plunger was used to hold the presure and control the feed rate. The cooking solution was collected manually by maintaining the pressure of the reactor around 450 psig.

The cooking solution was analyzed by using a high performance liquid chromatograph (HPLC) equipped with an Aminex HPX-87H organic acid analysis column (Bio-Rad Laboratories, Richmond, CA) and a refractive index detector (Waters Associates, Milford, MA). The operating conditions were as follows: eluent, 0.01N H_2SO_4 solution; column temperature, 65°C; and eluent flow rate, 0.5 ml/min.

Anaerobic Fluidized-Bed Digester

The anaerobic fluidized-bed digestion process has been developed to test the digestibility of the cooking liquor. Figure 2 gives the overall process scheme which consists of (1) water jacketed fluidized-bed bioreactor, (2) gas-liquid separator, (3) temperature controlled water circulator, (4) recirculation pump, (5) gas collector, (6) 0.2 μm membrane filters for sterization, and (7) feed tank as well as feed pumps.

Two bioreactors were employed: Reactor #1 is made of plexiglass with an inside diameter of 3.5 cm and a height of 75.2 cm; and Reactor #2 is made of glass with an inside diameter of 5.0 cm and a height of 60.0 cm. Each reactor has a water jacket which was used to maintain the operation at 35°C. Sand particles with an average

diameter of 1 mm were used as supporting carriers which allowed the bacteria to grow and develop as biofilms. The reactor configurations, reactor volumes, reactor free volumes, and operating conditions are listed in Table 1. The reactor free volume includes the reactor volume which is unoccupied by the sand particles and the volume in the recirculation loop. This value is used to evaluate the hydraulic retention time.

Medium solution was sterilized through two consecutive membrane filters and stored in the feed tank at room temperature. The feed tank, membrane filters, and associated silicon tubing were autoclaved before loading the substrate to the feed tank. Nitrogen gas was bubbled through the feed tank to reduce the dissolved oxygen in the medium solution. A dripper was placed between the feed tank and the feed pump to prevent the medium from back contamination. The produced biogas was collected and measured by using a gas collector. The acidified deionized water having a pH of 2.0 was used to avoid the dissolution of carbon dioxide in water.

The digester was seeded with the sewage sludge from an anaerobic digester in the municipal waste treatment plant in West Lafayette, Indiana. The culture was developed and acclimated for several months by using the synthetic feed which is similar to the composition of cooking liquor. The synthetic feed was injected daily in a fed-batch mode. After that, the cooking solution was fed to the digester continuously. To keep the bacteria growing well, the following nutrients and buffer were also added: potassium phosphate (0.2 g/ℓ), ammonium chloride (0.5 g/ℓ), sodium bicarbonate (1.0 g/ℓ), yeast extract (0.1 g/ℓ), and sodium sulfite (0.1 g/ℓ).

The volatile organic acids in the effluent were analyzed using the same HPLC system mentioned before. The chemical oxygen demand (COD), which is used to evaluate the performance of the anaerobic digester, was determined by the dichromate reflux method given in ASTM 1252D. Sludge samples were centrifuged at 10,000 rpm for 15 minutes to remove suspended solids before analysis. The pH values of the influent and effluent of the digester were also measured by a pH meter.

RESULTS AND DISCUSSION

Chemical Pretreatment

It has been well documented that cellulosics, such as wood and paper, and food waste are the two most abundant components found in the municipal solid wastes [Blum 1976; Snyder 1974]. Cellulosics represents about 65% of organic MSW on the basis of dry weight, while food waste represents 25%. Food waste, such as starch, can be complete solubilized by alkaline solution at a temperature of 170-200°C. This implies that the alkali pretreatment of MSW is rate-controlled by the degradation of cellulosics. Therefore, we investigated the alkali pretreatment of three model substances in which sawdust represents the untreated cellulosics, waste paper represents the treated cellulosics, and pure cellulose represents the refined product from the cellulosics.

Figure 3 shows the time courses of overall conversion of 10 wt% pure cellulose at temperatures ranging from 235°C to 300°C. The experiments were carried out in microtubular reactors and an excess amount of calcium hydroxide was added to remove the situation of complete depletion of calcium hydroxide during the reaction. The time courses, shown in Figure 3, cannot be fitted by simple power law model because the

reaction proceeds very fast in the initial period and slows down drastically as time goes on. The same conclusion is also held for the alkaline degradation of 18 wt% of pure cellulose (Figure 4).

The complete reaction mechanism of alkaline degradation of cellulose at 250°C to 300°C is still not well understood [Molton and Demmitt 1978; Richards 1963]. At a relatively low temperature (< 100°C), Machell and Richards [1953] showed that the alkaline degradation of cellulose is mostly from the reducing end group which results in a progressive decrease of degree of polymerization of cellulose molecule. However, the reaction may be stopped by such a competing process which converts the reducing end unit to a glucometasaccharinic acid group while it is still attached to the cellulose molecule. Therefore, the degradation process stops when all of the reducing end groups have been converted to the glucometasaccharinic acid groups. The conversion of this degradation process can be further enhanced by increasing the temperature up to 170°C under which the scission of glycosidic linkages occur [Richards 1963]. This results in new reducing end groups which are subject to the same degradation process at the lower temperature. When the reaction temperature increases above 250°C, the reaction mechanism becomes more complicated. It is suspected that such a high reaction temperature may accelerate the scission reaction which produces more new reducing end groups for the further fragmentation reactions producing low molecular weight salts of organic acids. If that is really so, the high conversion of the degradation process at 250°C to 300°C and the low degradation rate after the initial reaction period can be explained.

The decreasing rate of alkaline degradation of cellulose with reaction time also may be partly attributed to the low solubility of calcium hydroxide in the reaction medium. The solubility of calcium hydroxide in the mixture decreases with increasing temperature, showing a quite low solubility (< 0.0013 g/1000 g H_2O) at 250°C to 300°C. This implies that the dissolution of calcium hydroxide as well as the diffusion of hydroxide ion onto the cellulose particle might be the rate controlling step after the reaction proceeds for a short period.

Figure 5 gives the time courses of overall conversion of alkaline degradation of sawdust (black oak) and waste paper. Sawdust is more difficult to degrade than pure cellulose; however, waste paper solubilizes as fast as pure cellulose. Therefore, it can be concluded that the wood product is more easily degraded than the wood itself.

The composition of the cooking liquor were determined by HPLC. Four organic acids such as lactic acid, glycolic acid, acetic acid, and formic acid are identified; however, several peaks remain unknown. These peaks did not appear to be oxalic acid, levulenic acid, succinic acid, propionic acid, butyric acid, ethanol, methanol, acetone, or methyl ethyl ketone. The HPLC conditions we employed can separate lactic acid and glycolic acid very well as compared with the conditions used by Krochta et al. [1984].

At 250°C to 300°C, the main products in alkali-cooking appears to be acid formation to form salts with the added alkali. Figures 6 and 7 show the time courses of acids production in the alkaline cooking of 10 wt% and 18 wt% of pure cellulose, respectively. Among these four volatile acids, lactic acid is the main product and glycolic and acetic acids are the least. However, acetic acid becomes the second most abundant product in the alkaline degradation of sawdust, as shown in Figure 8. This is because of about 30 wt% of hemicellulose contained in the sawdust. It is believed

that the alkaline degradation of cellulose produces mainly lactic acid while the alkaline degradation of hemicellulose produces lactic acid as well as acetic acid.

At a higher temperature, in the range of 300°C or above, carbon dioxide formation is accelerated causing carbon loss. Also, lactic acid is significantly decreased as the reaction proceeds for longer than 15 minutes (Figures 6(c) and 7(c)). This shows that decarboxylation of the organic acids to the formation of alcohols and ketones starts to become predominant at such a high temperature. For the purpose of production of water-soluble organic compounds from the cellulosics, the reactions toward the production of alcohols and ketones is not what we want because of carbon loss. Hence, the experimental conditions are recommended to be carried out on the left-hand side of the dash lines shown in Figures 3 and 4.

Table 2 gives experimental results of alkali-cooking of 10 wt% pure cellulose from the pilot-scale tubular reactor. Three fractions are collected in each run, in which the second fraction was the steady state data. The first fraction was the mixture of the cooking liquor and the preoccupied distilled water inside the reactor. The third fraction was the partially reacted cooking solution which was obtained by flushing the rest of the reaction mixture inside the reactor very quickly to prevent the cellulose particles from sticking on the reactor wall.

The overall conversion was determined by the COD of the cooking liquor. It can be seen that the COD value of the second fraction in each run is close to those obtained from the micro-tubular reactors and preparative tubular reactors. The scale-up of chemical pretreatment seems to be less troublesome as compared with the anaerobic digestion process. The data obtained from the micro-tubular reactor can be used to predict the reactor performance of the pilot-scale reactor if the cellulose particles are completely wetted by water before cooking and heat transfer is not a problem. In short, this chemical pretreatment is largely dependent on the reaction temperature and the existence of alkaline solution in the reaction mixture. The presence of water is essential; however, the amount is not critical [Chesley et al., 1956], as the cases shown in Figures 3 and 4.

Anaerobic Fluidized-Bed Digester

The digestibility of the cooking liquor to methanogenics was tested on two anaerobic fluidized-bed digesters by varying either the feed substrate concentration (COD) or the feed rate. Both parameters can be combined and expressed by a single term, organic loading rate, which is the total amount of COD loaded per unit free reactor volume per day. The reactor performance can be expressed by the following two indices: % conversion, and gas production rate per gram of COD removed. The % conversion is defined as

$$\%\ conversion = (1 - \frac{[C_o]}{[C_i]}) \times 100 \qquad (1)$$

in which $[C_i]$ and $[C_o]$ represent concentrations of cooking liquor, in terms of gram COD/ℓ, in inlet and outlet liquid streams, respectively.

Typical experimental results from Reactor #1 and Reactor #2 are shown in Figures 9 and 10, respectively. The figures record the daily experimental results which include hydraulic retention time, organic loading rate, pH values of effluent, gas production rate, % conversion, gas production efficiency, and organic acids

concentrations. It shows that the conversion can be higher than 90% for both reactors if the organic loading rate is below 2.0 g COD/ℓ /day and the hydraulic retention time is between 1.5 and 2.0 days.

The composition of cooking liquor fed to each reactor is quite different; however, the digestibility is almost the same. For Reactor #1 the substrate was from the cooking liquor obtained from preparative tubular reactors under the conditions of 275°C and 30 minutes; for Reactor #2 the substrate was from the pilot-scale tubular reactor under the conditions of 240°C and 13-18 minutes. In both cases, 10 wt% of pure cellulose was employed. As shown in Figures 9 and 10, higher than 90% conversion is attainable for both reactors, suggesting that the digestibility of cooking liquors from various cooking conditions has almost no discrepancy under the experimental conditions investigated.

The effluent was analyzed by HPLC to identify intermediates produced in the methane fermentation. The observed fermentation products in the liquid effluent include acetate, propionate, butyrate, and a trace amount of ethanol. A high organic loading rate increases the acids production, which largely decreases the reactor performance in terms of % conversion. At the low organic loading rate, butyrate and ethanol disappear and acetate and propionate decrease to a trace amount. These observations imply that the rate limiting step of methane fermentation for high organic loadings is in the stage of converting the acids to methane.

Modeling of the fluidized-bed digester is complicated because the bacterial cells exist everywhere inside the reactor as well as in the recirculation loop. The cells are not only held by attachment to the carrier support, reactor wall, separator wall, and pipe wall, but also suspended in the bulk liquid. Also, the total cell mass and the bacteria population varies as either substate composition or recirculation flow rate changes. In such a case, fluid hydrodynamics play an important role in determining the relative ratios of cell mass in different supports as well as in the bulk liquid. The fraction of cell mass in the bulk liquid should be minimized because the methanogenic bacteria grow very slowly with doubling time ranging from 2 to 36 hours. Operating the reactor at low hydraulic retention time may result in low reactor performance if the fraction of cell mass in the bulk liquid is high. Therefore, the reactor configuration, the recirculation inlet nozzles, and the shape and size of supporting carrier become important factors in minimizing the drag forces exerted on each supporting particle and decreasing the collision frequency of supporting particles. A lighter and porous supporting carrier may be employed to decrease the energy consumption in the reactor operation and, perhaps, to increase the solid (bacteria) retention time.

FUTURE WORK

1. A small extruder and accessories have been ordered. After installation, the system will be used to process alkali cooking of MSW in a continuous manner with a much closer controlled reaction time as well as temperature.

2. New solid carriers are being tested for cell immobilization in the fluidized methane biogenerator. Several types of polymeric pyridine particles have been found to enhance greatly the cell loading as well as the hydrodynamic behavior in the reactor.

3. A complete unit of a small scale processing system will be in place once (1) and (2) are satisfactorily completed. The unit can be used to demonstrate the combined chemical and biological process for methane generation from MSW. It can also be used to test its application in conversion of various types of industrial and municipal solid wastes.

4. The alkali cooking generates a lot of organic acids, particularly lactic acid. The polymeric pyridine particles can adsorb and thus recover the organic acids. If the methane price continues to be depressed, the recovery of the higher valued acids can be further pursued.

CONCLUSION

The technical feasibility of processing cellulosic waste materials for methane production by a combined chemical and biological process was investigated. The major portions of the process, i.e., alkaline degradation of cellulosics and anaerobic digestion of the cooking liquor, were shown to be feasible for further consideration of scale-up and process design.

ACKNOWLEDGEMENTS

The authors wish to acknowledge support by the U.S. Department of Energy through a contract from the Argonne National Laboratory (contract number 40512401).

REFERENCES

Blum, S. L. 1976. "Tapping Resources in Municipal Solid Waste," *Science* 191.

Chesley, K. G., Montgomery, C. W., and Sandborn, L. T. 1956. " Production of Organic Acids and Salts Thereof from Cellulosic Materials," U. S. Patent, 2,750,414.

Clausen, E. C., and Gaddy, J. L. 1984. "High Solids Digestion of MSW to Methane by Anaerobic Digestion," Proceedings of the First Symposium on *Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals*, Minneapolis, MN, August 15-17, 283.

Colleran, E., Barry, M., and Wilkie, A. 1982. "The Application of the Anaerobic Filter Design of Biogas Production from Solid and Liquid Agricultural Wastes," in Symposium papers, *Energy from Biomass and Wastes VI*, Institute of Gas Technology, Chicago, IL, 443.

Haggin, J. 1987. "Efforts Intensify to Convert Methane to Fuels Directly," *C&EN* June 1, 22.

Haggin, J. 1987. "Catalytic Routes Probed for Converting Methane to Fuel," *C&EN*, June 8, 20.

Jeris, J. S., and P. L. McCarty, P. L. 1965. "The Biochemistry of Methane Fermentation Using C14 Tracers," *JWPCF* 37:178.

Krochta, J. M., Hudson, J. S., and Drake, C. W. 1984. "Alkaline Thermochemical Degradation of Cellulose to Organic Acids," *Biotech. Bioeng. Symposium* 14:37.

Machell, G., and Richards, G. N. 1953. "The Alkaline Degradation of Polysaccharides, Part II. The Alkali-Stable Residue from the Action of Sodium Hydroxide on Cellulose," *J. Chem. Soc.* 4500.

Molton, P. M., and Demmitt, T. F. 1978. "A Literature Survey of Intermediate Products Formed During the Thermal Aqueous Degradation of Cellulose," *Polym.-Plast. Technol. Eng.*, 11(2):127.

Richards, G. N. 1963. "Alkaline Degradation," *Methods in Carbohydrate Chemistry*, Vol. III, Academic Press, NY, 154.

Snyder, N. W. 1974. "Energy Recovery and Resource Recycling," *Chem. Eng.*, Deskbook Issue, Oct. 21.

Tsao, G. T. 1984. "Bacterial Hydrolysis: A Review," in *Anaerobic Digestion and Carbohydrate Hydrolysis of Waste,* edited by G. L. Ferrero, M. P. Ferranti, and H. Naveau, Elsevier Applied Science Publishers, London, England, 83.

Yang, S. T., and Chang, M. 1985. "Solubilizing Cellulosic Materials by Alkali-Cooking for Anaerobic Methane Production," *Annual Reports on Fermentation Process* 8:187.

Table 1. Experimental Conditions of Two Anaerobic Fluidized-Bed Digesters

	Reactor #1		Reactor #2	
Material	Plexiglass		Glass	
Sand Loaded	600 grams		1000 grams	
Reactor Volume	820 c.c.		1250 c.c.	
Total Free Volume	1500 c.c.		1950 c.c.	
Temperature	35°C		35°C	
Sand Bed Volume				
Before Expansion	300 c.c.		500 c.c.	
After Expansion	400 c.c.		650 c.c.	
Feed				
Cooking Liquor	10% cellulose		10% cellulose	
Operating Time	275°C; 30 minutes		240°C; 13-18 minutes	
	(Days)			
	1-50	Synthetic substrate	1-23	Cooking liquor; sterilization (membrane); nitrogen bubbling
	91-185	Cooking liquor; no sterilization	23-32	Cooking liquor; sterilization (membrane); nitrogen bubbling
	186-203	Cooking liquor; no sterilization; nitrogen bubbling	(Operating time is counted from the day of taking data)	
	204-210	Cooking liquor; heat sterilization; nitrogen bubbling		
	211-232	Cooking liquor; sterilization (membrane); nitrogen bubbling		

Table 2. Experimental Results of Alkali-Cooking from the Pilot-Scale Tubular Reactor

Run No.	Residence Time (Minutes)	Fraction	Volume (liter)	pH	COD (g/liter)	Lactic Acid (g/L)	Glycolic Acid (g/L)	Formic Acid (g/L)
1	13.0	First	0.83	11.94	9.24	3.72	0.53	1.64
		Second	2.40	12.34	36.71	8.47	1.07	3.28
		Third	3.54	12.43	10.59	4.64	0.63	1.85
2	18.0	First	1.16	11.80	17.06	3.896	0.50	1.70
		Second	1.50	12.29	35.82	7.84	1.03	3.41
		Third	4.00	12.10	8.33	1.39	0.21	0.64

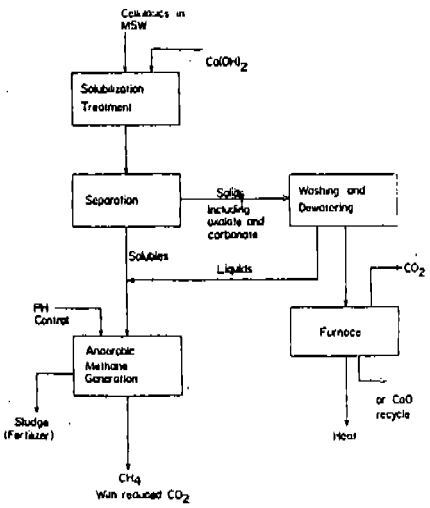


Figure 1. Process outline involving alkali-cooking.

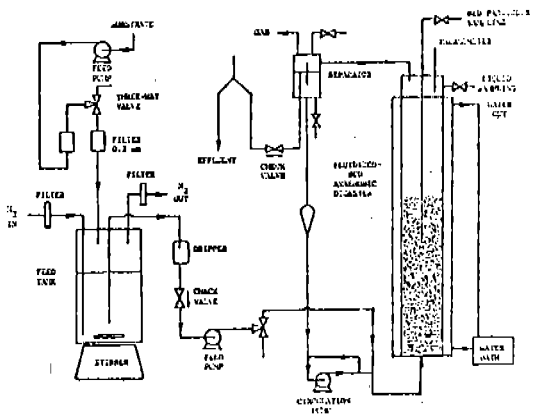


Figure 2. Scheme of the anaerobic fluidized-bed digester.

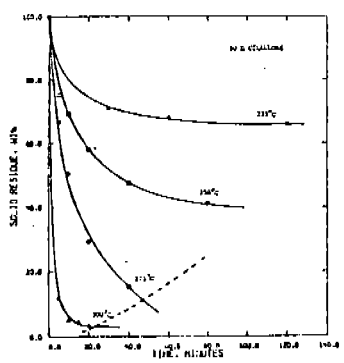


Figure 3. Time courses of alkali-cooking of 10 wt% pure cellulose.

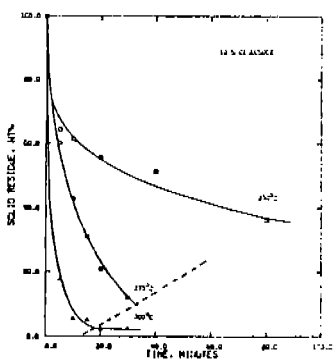


Figure 4. Time courses of alkali-cooking of 18 wt% pure cellulose.

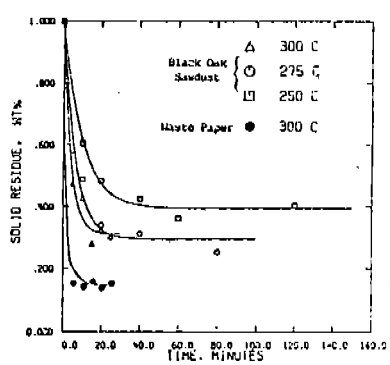


Figure 5. Time courses of alkali-cooking of 10 wt% black oak sawdust (50 mesh) and 10 wt% waste paper.

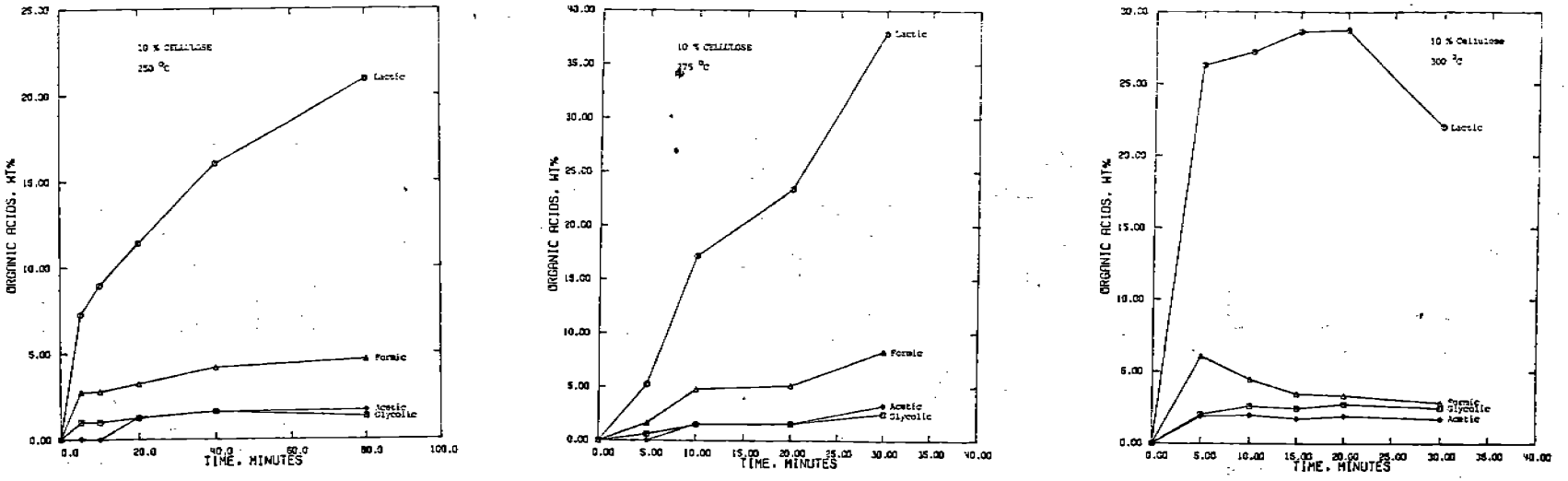


Figure 6. Acid products from 10 wt% cellulose after alkali-cooking.

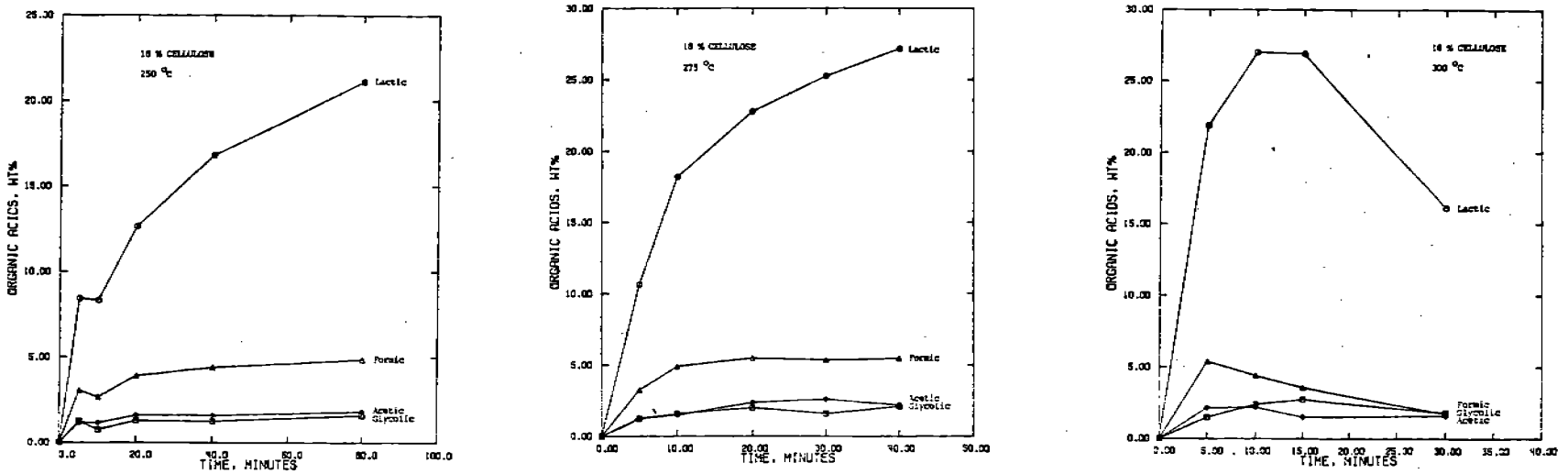


Figure 7. Acid products from 18 wt% cellulose after alkali-cooking.

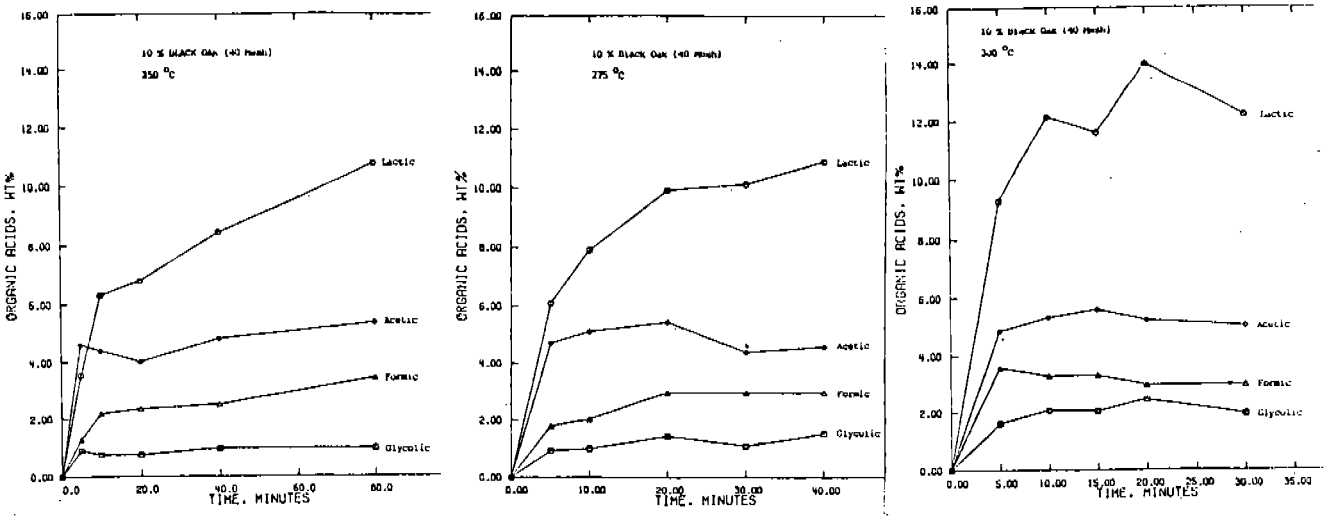


Figure 8. Acid products from 10 wt% black oak sawdust after alkali-cooking.

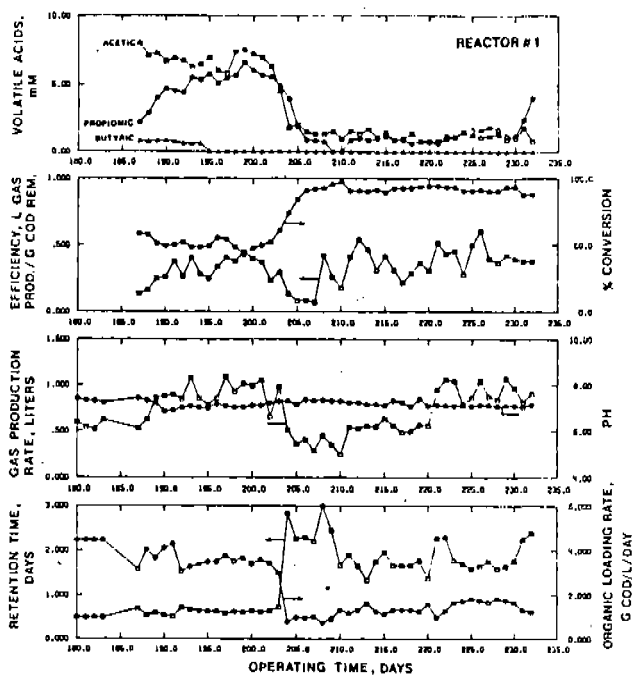


Figure 9. Performance data for the anaerobic fluidized-bed digester (#1) with the alkali-cooking liquor.

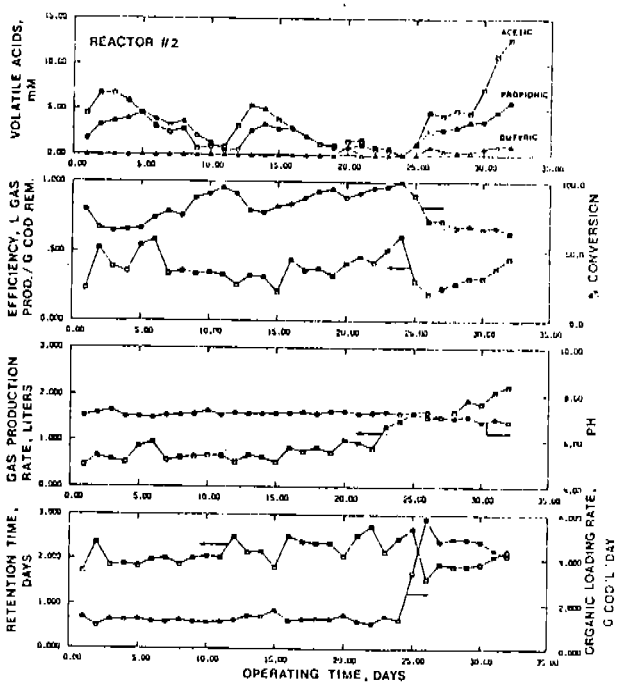


Figure 10. Performance data for the anaerobic fluidized-bed digester (#2) with the alkali-cooking liquor.

ANAEROBIC HIGH SOLIDS FERMENTATION OF PROCESSED MUNICIPAL SOLID WASTES FOR THE PRODUCTION OF METHANE

Christopher J. Rivard, Michael E. Himmel, Todd B. Vinzant,
William S. Adney, Charles E. Wyman, and Karel Grohmann
Biotechnology Research Branch
Solar Energy Research Institute
Golden, Colorado 80401

ABSTRACT

A novel reactor that can process high solids concentrations is described for the anaerobic fermentation of lignocellulosic material, specifically, processed municipal solid waste. The reactor has a total volume of approximately 20 liters and is hydraulically driven. The solids in the reactor are mixed by a blade with a horizontal axis. Several different mixing blade designs were tested on uninoculated solids for mixing quality and horsepower requirement, and the blade design requiring the least power input was selected for further study. The reactor has been inoculated with solids, and initial production of methane is reported.

ANAEROBIC HIGH SOLIDS FERMENTATION OF PROCESSED MUNICIPAL SOLID WASTES FOR THE PRODUCTION OF METHANE

INTRODUCTION

Anaerobic digestion of municipal solid waste (MSW) couples the potential of producing considerable energy (methane) with the simultaneous reduction in organic disposal problems by a much less energy intensive process than conventional aerobic methods. However, several key issues must still be overcome before the methane produced is economically competitive with conventional sources of natural gas. Since the value of the gas produced, methane, is relatively low, the anaerobic process must be rather simple in design, require little energy to operate, and have high gas production rates.

Economics evaluations of anaerobic digestion for production of gas from municipal solid waste show that reactor capital costs are a primary economic factor due to the large reactor volumes required in current anaerobic processes. If the reactor volume could be reduced significantly, the economics of anaerobic digestion would improve. Increased solids loadings are particularly promising in this respect since available kinetic data indicate that gas production rates should increase with solids concentration in the reactor. Thus, if this data is substantiated, a decreased reactor volume would be possible for higher solids concentrations while maintaining the same solids loading rate and retention time. However, high solids slurries are very viscous and resemble solid materials more closely than typical fluids. Therefore, conventional mixers do not insure homogeneity in the reactor, and problems develop in providing adequate dispersion of intermediates and microorganisms.

Research on high solids anaerobic fermentation has focused on the single charge, batch, non-mixed reactor concept, generally with recirculation of effluent (Ghosh 1984, Goebel 1983, Jewell et al 1980, Lin 1983, SEG 1983, Wujcik and Jewell 1979). Gas production occurs either in a single stage or leached acids may be circulated to a second methanogenic stage. With non-mixed systems, rates of gas production are generally much slower than liquid mixed systems. The retention time required to effect a near complete digestion of the substrate is on the order of months for mesophilic temperatures and may be as short as weeks for thermophilic operation.

Two research efforts were devoted to a mixed high solids fermentation design. The "Bio-Funnel" (Goldberg et al 1981) was used with 21-13% solids, while Gaddy and Clausen (1986) attained high solids feeding in a modified CSTR which approached 10% solids within the digester. Reactor design options in which the high solids material within the reactor may be mixed, will increase the interaction of substrate, microorganisms, and metabolic intermediates. The effective mixing and operation of these high solids designs have resulted in problems with material movement due to bridging of substrate, scum formation when the solids levels fall to 10% or lower, and non-mixing zones within the reactor.

The high solids reactor designed, fabricated and employed in this project is a significantly different concept than previously used in high solids anaerobic digestion research. This reactor may be used in batch or continuous

operation, results adequate mixing of high solids materials (10-50%) in the reactor, and is based upon reactor designs conventionally used in the mixing of highly viscous materials in the plastics industry.

MATERIALS AND METHODS

High Solids Reactor Design and Construction

The reactor is a cylindrical glass vessel which is positioned with a horizontal axis, as shown in Figure 1. The cylinder is capped at each end, and a shaft is positioned horizontally along the cylinder axis between the centers of the two end caps. Several ports are added to the glass vessel for feed addition and sample removal, gas removal and liquid addition. Mixing is obtained with one of several different blade configurations attached to the horizontal shaft. Fabrication of the high solids reactors was by International Process Research Co., Golden, Colorado, and is shown in Figure 2. Shaft rotation is provided by a low speed, high torque, hydraulic motor (5 piston, 35 horsepower maximum, Staffa, England). The hydraulic motor is powered by a custom fabricated hydraulic power station (10 horsepower, Warren Fluid Power, Denver, CO). The Staffa hydraulic motor speed was controlled by Vickers temperature compensated flow control valves. The shaft seal was fabricated as a stuffing box type seal using teflon rope packing and provided a liquid/gas tight seal even at fractional rpm operation of the reactor.

Several blade configurations were designed, fabricated and tested for mixing ability and horsepower requirements (Figure 3). The final blade configuration chosen for fermentation studies (90° rod) was teflon coated to reduce biomass buildup and metal pitting from anaerobic reactions.

The glass vessel was modified with two 3/4 inch ports for liquid introduction and gas removal. A 2 inch ball valve (Harrington Plastics, PVC) was attached with couplings to the 2 inch glass port with beaded lip at the top of the glass vessel and was used for sampling the contents of the reactor. Several glass receiving vessels were constructed which coupled to the outside of the 2 inch ball valve and allowed gassing of the receiver vessel with oxygen-free nitrogen gas before sampling. A solids addition unit was fabricated using a 2 inch plunger to add dry MSW feed to the reactor through the 2 inch ball valve. Two gassing ports were installed on the solids feeder to allow outgassing of the dry MSW before addition to the reactor.

Horsepower Determinations

Determination of horsepower was calculated by standard methods from the specific motor volumetric throughput and monitored hydraulic back-pressure. Horsepower requirements were determined for several blade configurations with various milled materials.

High Solids Reactor Operation

In anaerobic fermentation studies, the 90° rod or rake blade was chosen.

The speed of mixing was maintained at approximately 10 rpm. Several different methods of initiating the fermentation were used and are described in the results section. In general, the 20-Liter vessel was outgassed with oxygen-free nitrogen gas prior to the introduction of inoculum to remove air. The gas production was monitored using one of the ports on the glass vessel connected to calibrated water displacement reservoirs. Sludge was removed from the reactor on a daily or bi-daily basis for pH, acid analysis, and microbial observation.

Enhanced Acetogen/Methanogen Digester

A 2-liter New Brunswick Multigen fermenter (1.5-liter working volume) was used for production of an enhanced acetogen/methanogen consortium. The reactor was magnetically stirred and temperature controlled at 37°C using heat tape (Briskheat), Omega temperature controller (model 601), and K-type thermocouple. A liquid feedstock was used for constant feed addition and pH control. The liquid feed contained yeast extract, 8g/l; K_2HPO_4, 50mM; and 10x trace mineral solution, 10ml/l (Balch et al 1979), as well as the following organic acids: formic, 18mM; acetic, 766mM; propionic, 76mM; n-butyric, 102mM; n-valeric, 4mM; iso-valeric, 2mM; and lactic, 182mM. The final pH of the organic acid/nutrient solution was approximately 4.2. A Markson (model 6300) pH/ORP controller with gel-filled electrode monitored the reactor pH and added the organic acid solution when the pH approached 7.4 using a masterflex pump (Cole-Parmer). Effluent from the reactor (~500 ml) was removed weekly and cells concentrated by centrifugation under a oxygen-free gas phase in 500 ml plastic centrifuge bottles at 5000 rpm for 30 minutes and 4°C, using a Beckman centrifuge (model RC-5B) and GSA rotor. The cell pellets were stored at 4°C until use.

Digester Inoculum

Sludge from a 7-liter working volume anaerobic digester (Chemap) served as inoculum for the high solids reactors. The digester was maintained on a 20 day retention time and temperature controlled at 37°C using heat tape around the glass reactor and an Omega temperature controller (model 601) with K-type thermocouple. The reactor was fed a processed, densified, municipal solid waste from Thief River Falls, MN., which was knife milled using a 1 mm rejection screen. The reactor was batch fed daily a 5% solids mixture with addition of a nutrient supplement to the milled MSW. The nutrient supplement contained the following components per liter of distilled water; yeast extract (Difco) 8g, K_2HPO_4 8.71g, 10x trace mineral solution (Balch et al 1979) 10ml. The final pH of the nutrient solution was adjusted to 7.5.

High Solids Sludge Analysis

Levels of volatile organic acids (C1-C5 iso and normal acids) were determined by gas-liquid chromatography (GLC). A model 5840A gas chromatograph (Hewlett-Packard, Palo Alto, California), equipped with a flame ionization detector, a model 7672A autosampler, and a model 5840A integrator (all from Hewlett-Packard), was used. The glass column (6 ft x 2mm) was packed with Supelco 60/80 Carbopack C/0.3% Carbowax 20M/ 0.1% H3PO4. The injection

port temperature and detector were maintained at 190°C. The oven temperature was maintained at 120°C. Nitrogen at a flow rate of 50ml/min was used as the carrier gas. The experimental samples were calibrated against a quantitative standard (Supelco) with each run. Experimental samples were prepared for analysis by centrifugation to remove suspended solids in 1.5 ml micro-centrifuge tubes in a microcentrifuge (Eppendorf) at room temperature for 15 minutes. The clarified samples were acidified by addition (1:1) with 1% v/v formic acid. Finally, the samples are loaded into autosampler vials, crimp caps attached and loaded into the autosampler for analysis.

Analysis of non-volatile organic acids in digester sludge effluent was accomplished by high performance liquid chromatography (HPLC). Experimental samples are clarified by centrifugation as described above with final removal of particulates by passage through a 0.2 micron Acrodisc disposable syringe filter (Gelman #4418). The samples were made 0.01 N with sulfuric acid and loaded into autosampler vials for analysis. The HPLC system consisted of a Beckman model 501 autosampler, Beckman model 110A pump, temperature controlled column (Eldex), Variable Wavelength Detector (detection at 210nm, Waters and Assoc.), and integrating recorder (Hewlett Packard, 3390). The column used was a Bio-Rad HPX-86 organic acids packed column. The column temperature was controlled at 45°C. The eluent was 0.01 N sulfuric acid in distilled water which was filtered (0.2 micron) and degassed before use. The flow rate of eluent was maintained at 0.5 ml/min. Samples were analyzed in conjunction with high purity standard mixtures of organic acids (Supelco). Analysis by HPLC identified both non-volatile and volatile and therefore served as a backup to the gas-liquid chromatography analysis.

Solids analysis of digester effluents was conducted using 1.0 gram weigh tins. A 20-30 grams of sludge sample was loaded into the preweighed tin and dried 48 hours at 45-50°C. The tin/dry sample were cooled to room temperature in a laboratory desiccator and weighed using a Sartorious balance (model 1684MB). Percent solids in the digester sludge was calculated on a weight/weight basis.

Gas Analysis

Production of biogas from high solids reactors was monitored on a daily basis (in conjunction with batch feeding) using calibrated water displacement reservoirs.

Biogas produced in the various digesters was analyzed for methane and nitrogen composition by gas chromatography. A Gow-Mac (model 550) gas chromatograph equipped with a thermal conductivity detector and integrating recorder was used. The injection port, oven, and detector were maintained at 100, 90, and 110°C respectively. Helium served as carrier gas. The chromatograph was calibrated with high purity gas standards (Matheson).

Microbiological Analysis

Observation of sludge samples from high solids reactors were conducted using wet-mount slide preparations with a Nikon Labophot microscope equipped with phase contrast/epi-fluorescent illumination and 1000x power. Methane

producing bacteria were detected by their unique auto-fluorescence upon excitation by the epi-fluorescent light source (narrow band width centered at 420 nm).

RESULTS

Initial testing of horsepower requirements for mixing of MSW milled to two different sizes employed various blade configurations, as shown in Figure 3. The effectiveness of mixing of the material was also assessed. In general, the curved and straight solid blade designs required the most horsepower for mixing (Figures 4-7). The more coarsely milled MSW (1/8 inch) required more horsepower than the finer milled material (1 mm). As the blade-vessel clearance was reduced, the horsepower required for mixing increased; this was due to pinching of the MSW in the reactor at the blade-vessel edge. During extended operation of the solid blades, the volume of MSW on the sides of the blades became uneven due to variations in the exact blade-vessel clearances of the two edges of the blades. This asymmetry lead to large pressure variations on the hydraulic motor and translated into large horsepower swings.

The mixing of a 30% solids MSW material with the solid blade configuration also formed balls of material due to tumbling of the MSW down the face of the solid blade. This clumping of material within the reactor resulted in unsatisfactory mixing performance with both of the solid blade designs. The rake blade configuration (essentially a rod with end wiper) did not form clumps of material in the reactor, but in comparison to the rod blade configuration, required more horsepower for mixing (Figure 8,9).

During the first inoculated run of the high solids reactor with the rod type blade, material adhered to the rods and hub. After teflon coating of the rods and hub, no significant adherence of material occurred. The teflon coating also resulted in a lower horsepower requirement as compared to a non-teflon coated rod blade (Figure 9). The angle and alignment of the rods also affected the required horsepower and effectiveness of mixing. In general, changing the angle from 180 to 120 to 90 degrees and opposing the rods rather than aligning each row, resulted in less lifting of material and more even mixing of solids. Less lifting of the material resulted in a lower horsepower requirement and dampened the high and low horsepower spikes. As a result, the 90^{o} teflon-coated, opposed-rod blade configuration was used in the inoculated solids, digestion experiments.

The first operational run of the high solids reactor was initiated by addition of 1 kg of 1/8 inch hammer milled MSW to the reactor which was outgassed with oxygen-free nitrogen for 2 hours. Anaerobically, 3 liters of sludge was added. Over a 4 hour period, the MSW became uniformly moist, and gas production occurred. After 3 days, the pH dropped to 5.13, and analysis of the gas produced indicated no methane production. The determination minor oxygen in the gas phase indicated insufficient outgassing during start-up, and the fermentation was terminated.

The results of fermentation run 2 are shown in Figures 10 and 11. The reactor was initiated as in run 1 but with more extensive outgassing to remove oxygen. The total gas production quickly peaked and dropped off dramatically

along with the pH. With the exception of lactic acid, the level of organic acids increased in general to a cumulative acid level of approximately 0.2 M. Without pH control, the pH fell to 5.25 after 7 days of operation, and the fermentation was terminated.

The performance of fermentation run 3 is shown in Figures 12 and 13. The reactor was initiated as in run 1 and 2 but with outgassing overnight to more completely remove oxygen entrapped in the dry MSW material. The pH shifts were adjusted using 0.5% urea (final concentration in reactor) and later sodium carbonate. It was apparent that while the urea effectively buffered the pH shift, the effects of the added concentration were for a time inhibitory to the microbial population. The later addition of sodium carbonate (6 g/l) demonstrated a less severe effect. The level of organic acids continued to increase during the fermentation, especially for acetic acid with a final total organic acid level of approximately 0.175 M of which 80% was acetic. The fermentation was terminated due to the excessive levels of acetic acid present.

The high initial levels of acids in the high solids reactor runs indicated the need to enhance the population of acetogens and methanogens in the digester sludge used to inoculate the reactor. A fermenter was initiated to produce an enhanced acetogen/methanogenic culture by controlled continuous addition of organic acids. The acids and their relative concentration were determined by the final acid pools for high solids runs 2 and 3. The gas production and acid pools for this reactor are shown in Figure 14. The resulting microbial population consisted of many different rod forms and autofluorescing rods and large clumps of sarcina under epi-fluorescent microscopy. The cells from this fermenter were harvested, refrigerated for storage, and added to the digester sludge upon inoculation of high solids run 4 and 5.

Current information on high solids run 4 (last 42 days) is shown in Figure 15. With the initiation of the run as described in the figure, the operation was severely unstable requiring substantial pH adjustments, and the contents were diluted to 12% solids by addition of digester sludge (first 45 days data not shown) to maintain the fermentation. The fermentation has now become more stable, and with addition of the nutrient solution, most parameters have stabilized. The digester is now being batch fed dry MSW to build up the solids concentrations.

With the recent addition of a second high solids fermenter, it has been possible to run two experiments at once. Information on high solids run 5 with the second system is shown in Figure 16. Rather than initiating the fermentation with an overabundance of substrate, digester sludge only was added with addition of dry MSW and liquid nutrient solution added on a near daily basis. All parameters appear to be stable with solids approaching 11% currently.

DISCUSSION

The high solids reactor as designed, equipped, and fabricated, has achieved flexible operation (as low as fractional rpm operation without stepping); easy gas collection, sludge removal, and feed introduction, and reliable operation, particularly due to teflon stuffing box seals. Several

blade configurations have been evaluated on the basis of average horsepower requirements and mixing effectiveness using two different milled MSW substrates. The most effective mixing was accomplished by rods spaced at 90° angles around the shaft, with no clumping of high solids materials, the lowest horsepower, and the least high/low horsepower spikes. A problem with solids attaching to the rods (and hub and not being effectively mixed) was solved by teflon coating the rods and hub.

In initial fermentation runs with the high solids, the reactors have demonstrated that initiating the fermentation at the high solids level by the addition of a great excess of substrate results in a severely unstable fermentation. Apparently this problem is due to extremely rapid buildup of acids with the high substrate concentration before enough methanogens are available to use the acids. To achieve a balanced digester consortium, adaption to the high solids level by gradual dry substrate addition may well be required. After this balanced population is attained, high solids fermentation may occur stably. Several agents which may be useful for pH adjustment during the fermentation were identified and tested, and the relative concentrations of agents were determined that would not substantially reduce gas production.

FURTHER RESEARCH

With the length of operation of each fermentation, it is imperative that more reactors are in operation to speed the gathering of information. Therefore, two additional high solids reactor will be put on line in January 1988 along with a computer data collection system and temperature controlled room.

The development of an anaerobic digestion consortium adapted to the high solids operation is of primary importance. Therefore, the runs currently in progress, numbers 4 and 5, will be gradually taken to high solids levels by the feeding the reactors with dry MSW. Also, the development of improved acetogenic and methanogenic populations, which will increase the fermentation stability, will continue. Nutritional requirements of the microorganisms at the high concentration of solids will be determined along with optimization of pH control strategies. The results for the high solids operations will then be compared to those possible with conventional fermentation to determine the extent of rate improvements by operating at the high solids concentration. If the data confirm the high solids fermenter to achieve higher gas production rates per unit volume, then research will be undertaken to evaluate the influence of solids concentration and agitation rate on digester performance to optimize the design.

REFERENCES

Balch, W.E., Fox, G.E., Magrum, L.J., Woese, C.R., and Wolfe, R.S. 1979. "Methanogens: Reevaluation of a Unique Biological Group," Microbiol. Rev. 43:260-296.

Gaddy, J.L., and Clausen, E.C. 1985. SERI Anaerobic Digestion Program 1984 Annual Report. SERI/PR-231-2691.

Ghosh, S., 1984. "Solid-Phase Methane Fermentation of Solid Wastes," Eleventh American Society of Mechanical Engineers: National Waste Processing Conference, Orlando, FL., June 3-6, 1984.

Goebel, R.P., 1983. High Solids Anaerobic Digester for Rural Use. USDOE Technical Report #DE-FG03-81 SF 11613.

Goldberg, T., Bjergbaek, B., Djernaes, E., and Mouritzen, A. 1981. "An Anaerobic Digester for Continual High Solid Loading: The Bio-Funnel (A Radially Expanding, Overflow Digester)," International Gas Research Conference, 1981.

Jewell, W.J., 1980. "Anaerobic Fermentation of Agricultural Residue, Potential for Improvement and Implementation," Volume II USDOE Project #DE-AC02-76ET20051.

Lin, Y.-M., 1983. "Coupled High Solids Fermentation and Anaerobic Filtration of Cellulosic Residues," Ph.D. Thesis, Michigan State University.

SEG (Snell Environmental Group), 1983. "Rapid Methane Generation from Solid Waste," USDOE Technical Report #DE-FG02-81R510329.

Wujcik, W.J., and Jewell, W.J. 1979. "Dry Anaerobic Fermentation," 2nd Symposium on Biotechnology in Energy Production and Conservation, Gatlinburg, TN., October 3-5, 1979.

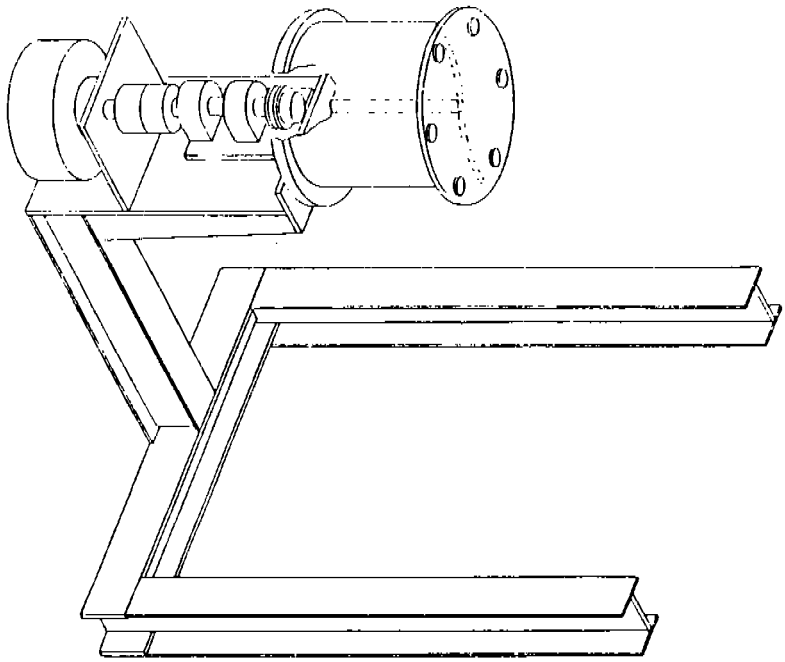


Figure 1. Drawing of the high solids reactor system with movable stand. The blade shown in this figure is the curved option.

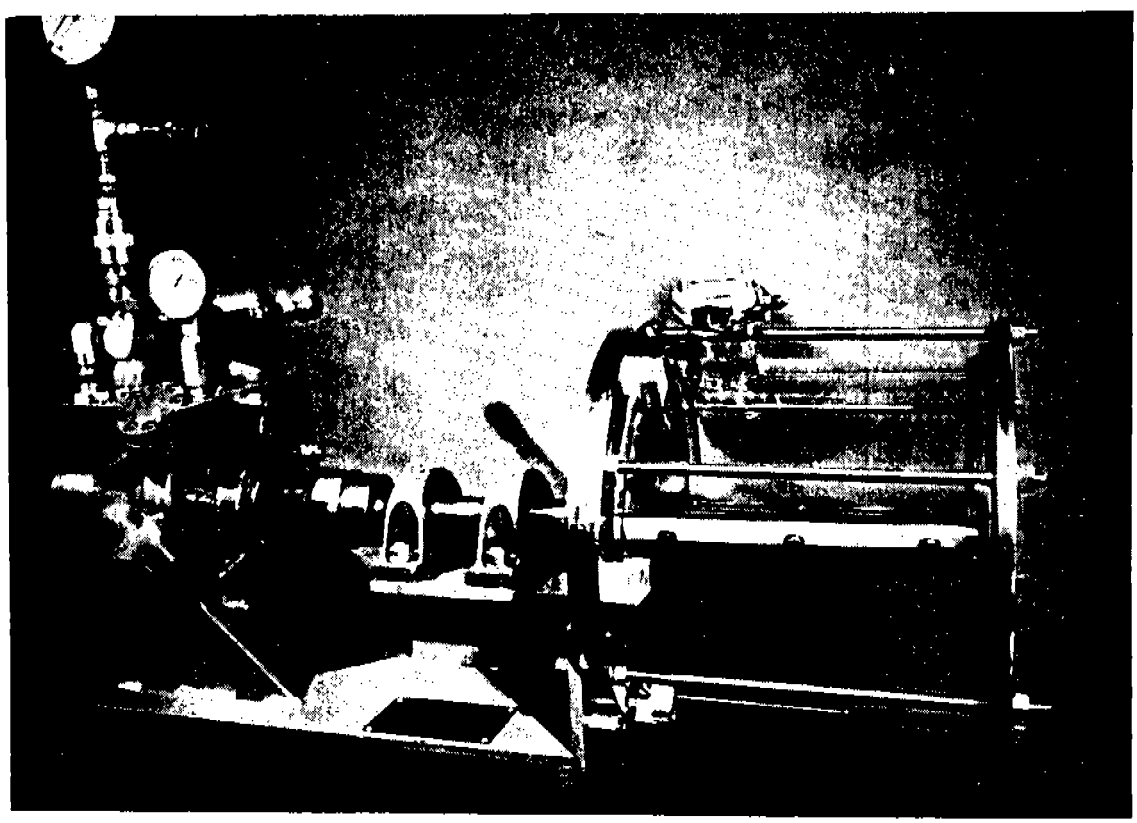


Figure 2. The bench scale high solids digester installed in the SERI FTLB high bay area. This picture shows the transparent vessel and the agitator blade along with the hydraulic motor.

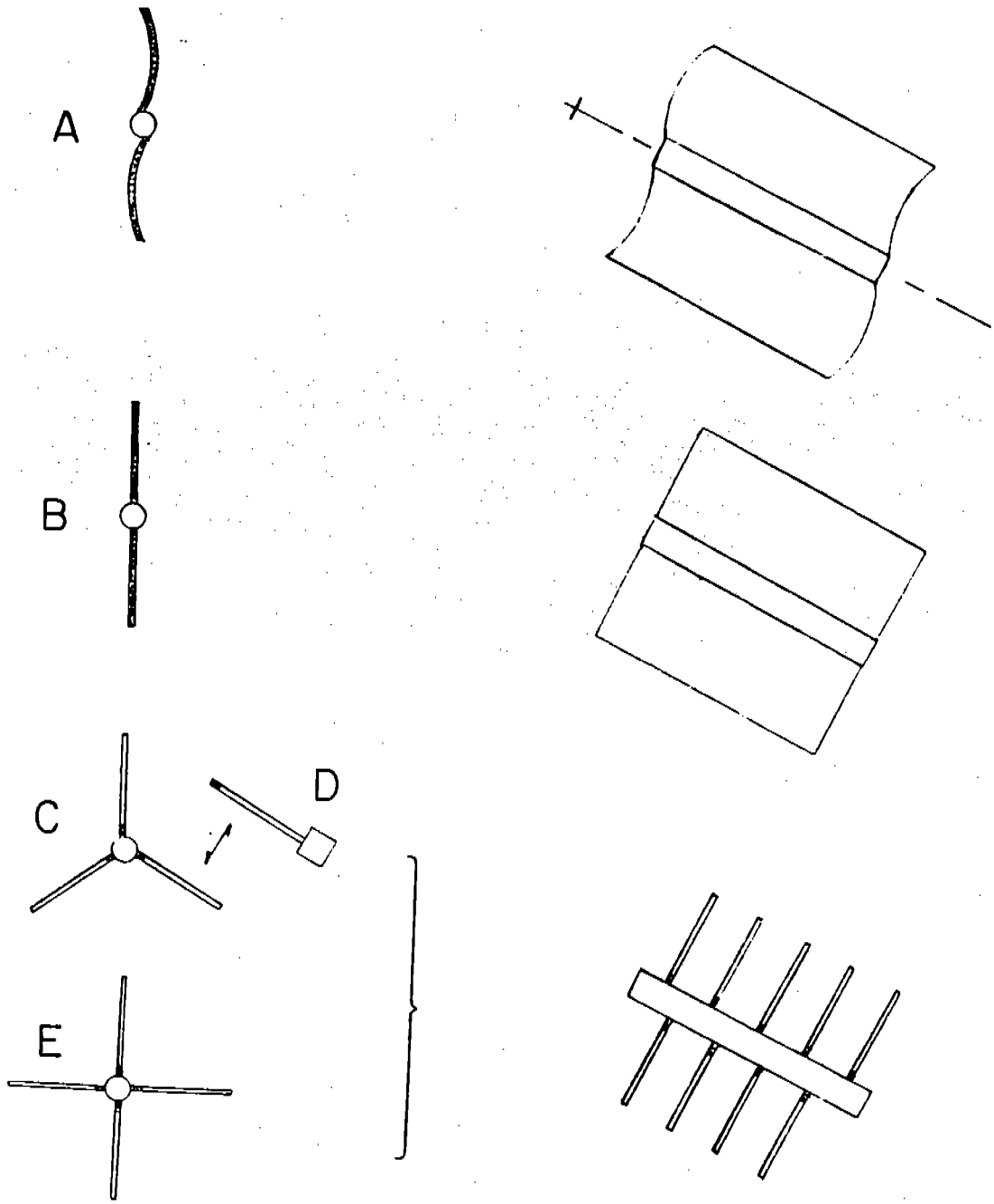
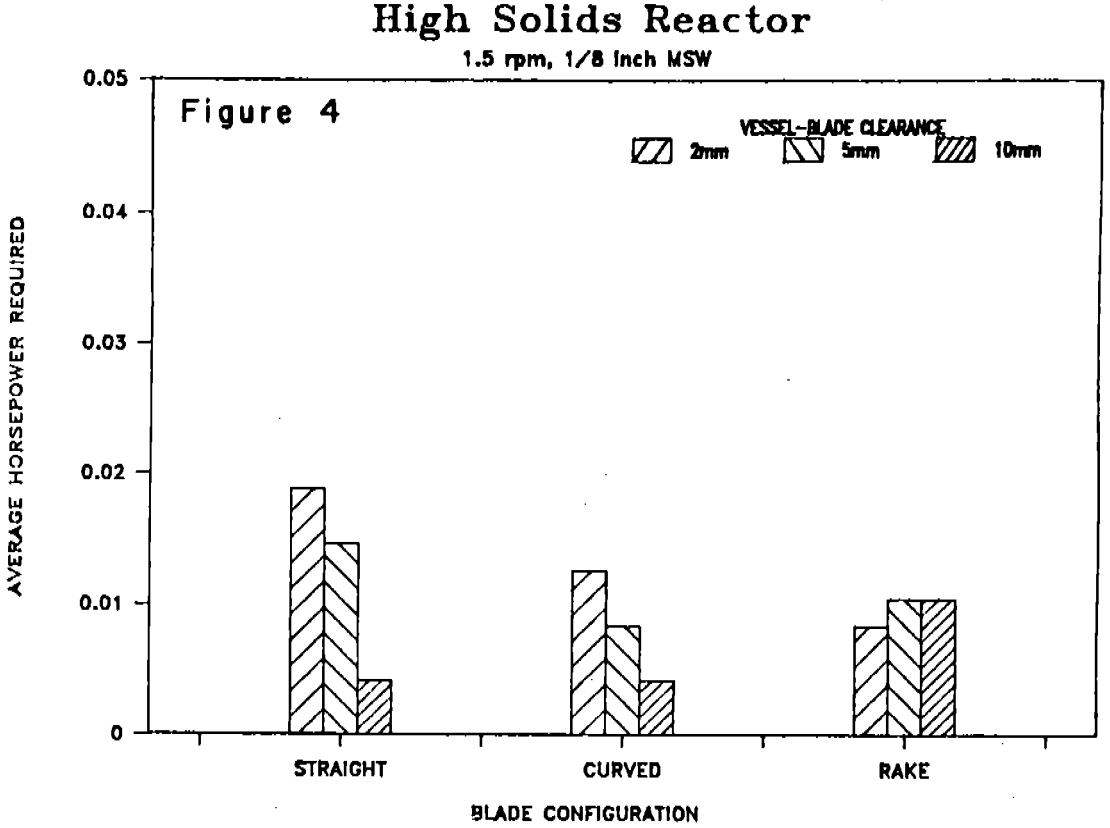


Figure 3. Blade configurations for high solids reactor mixing, curved (gull wing) (a), straight (b), 120° rod (c), 120° rake (d), and 90° rod (e).



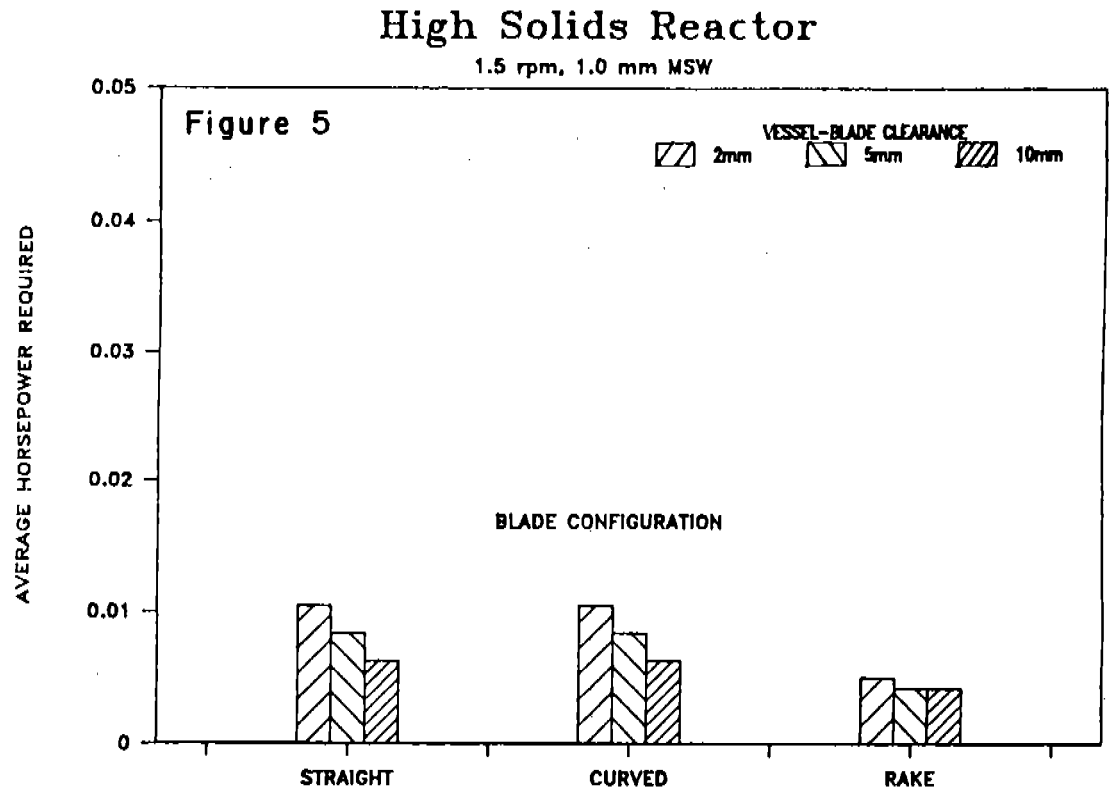
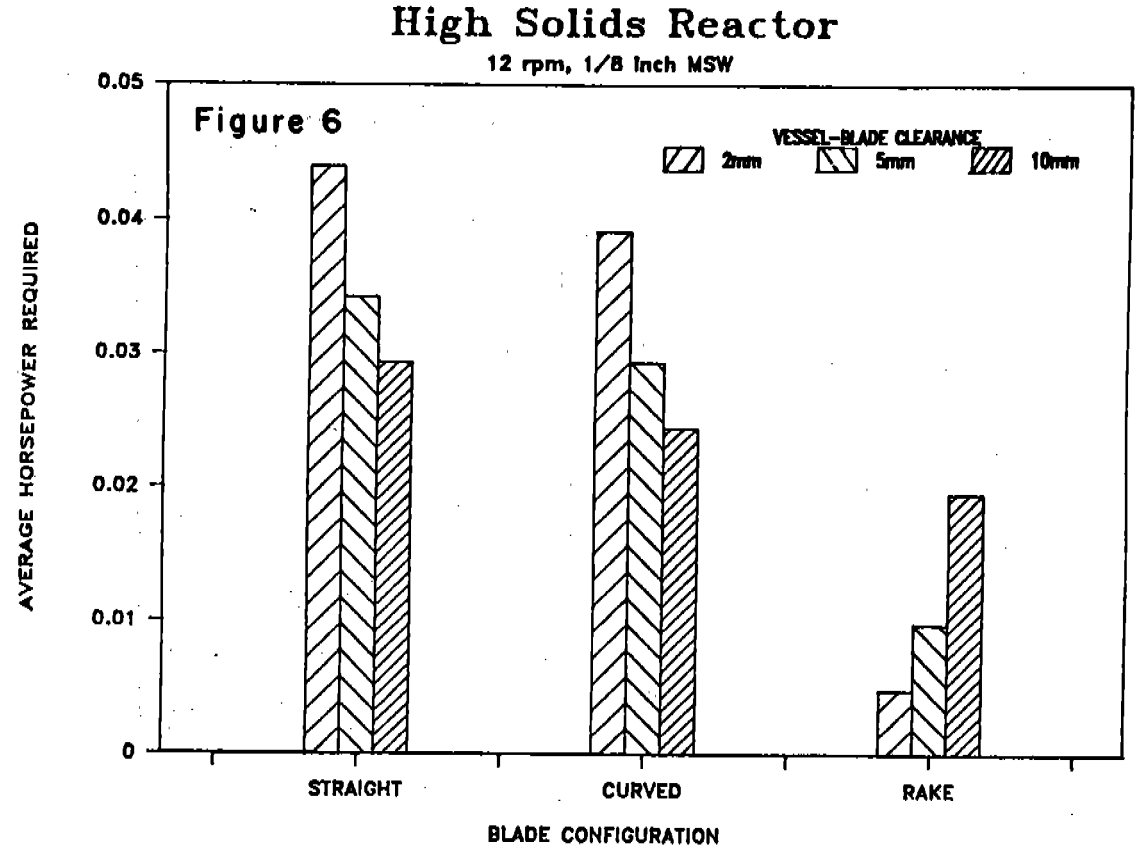


Figure 4 and 5. Average horsepower required for 1.5 rpm mixing of two different size milled MSW with various blade configurations and blade-vessel clearances.



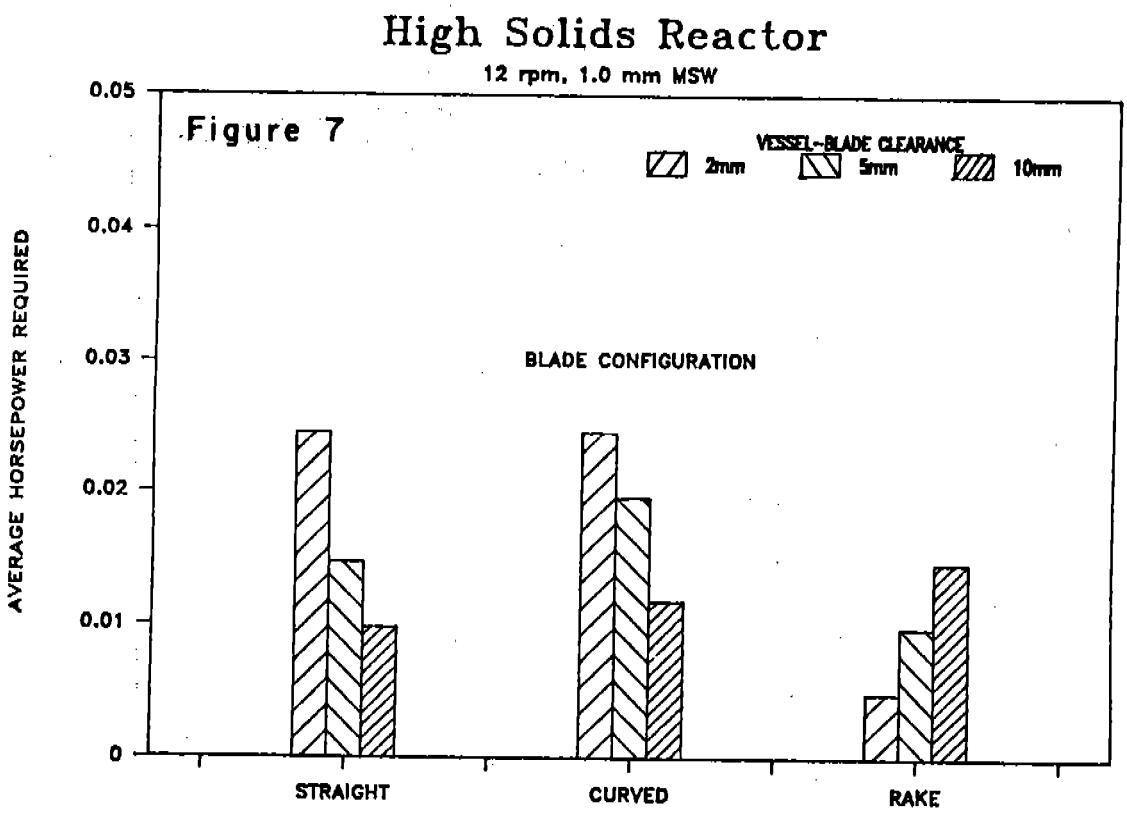
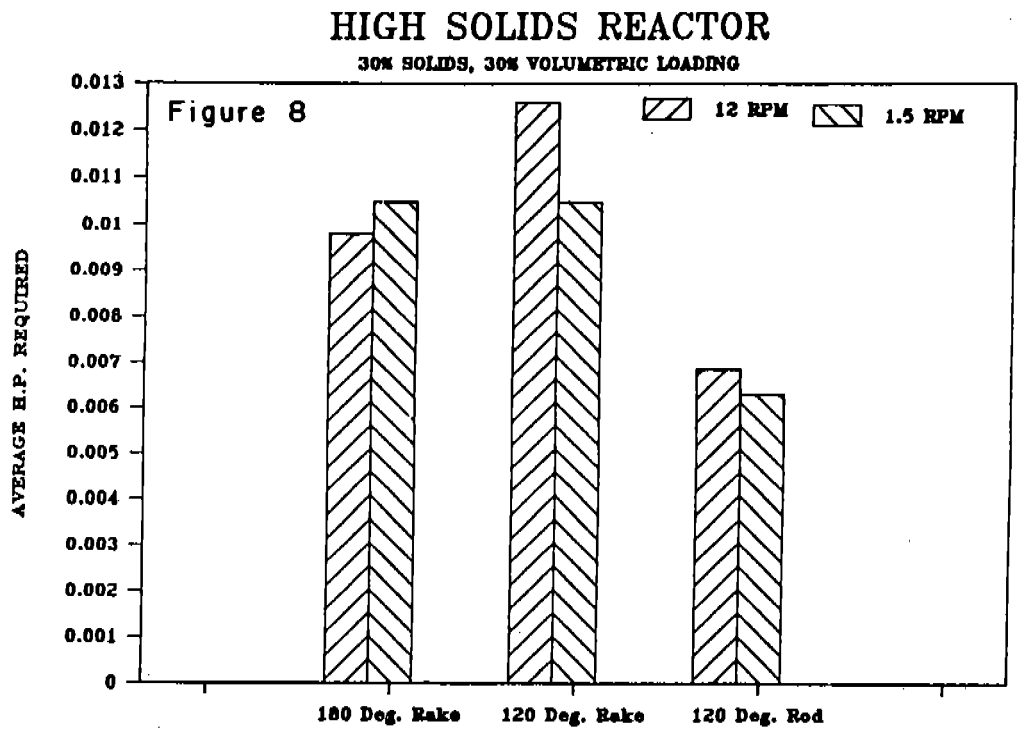


Figure 6 and 7. Average horsepower required for 12 rpm mixing of two different size milled MSW with various blade configurations and blade-vessel clearances.



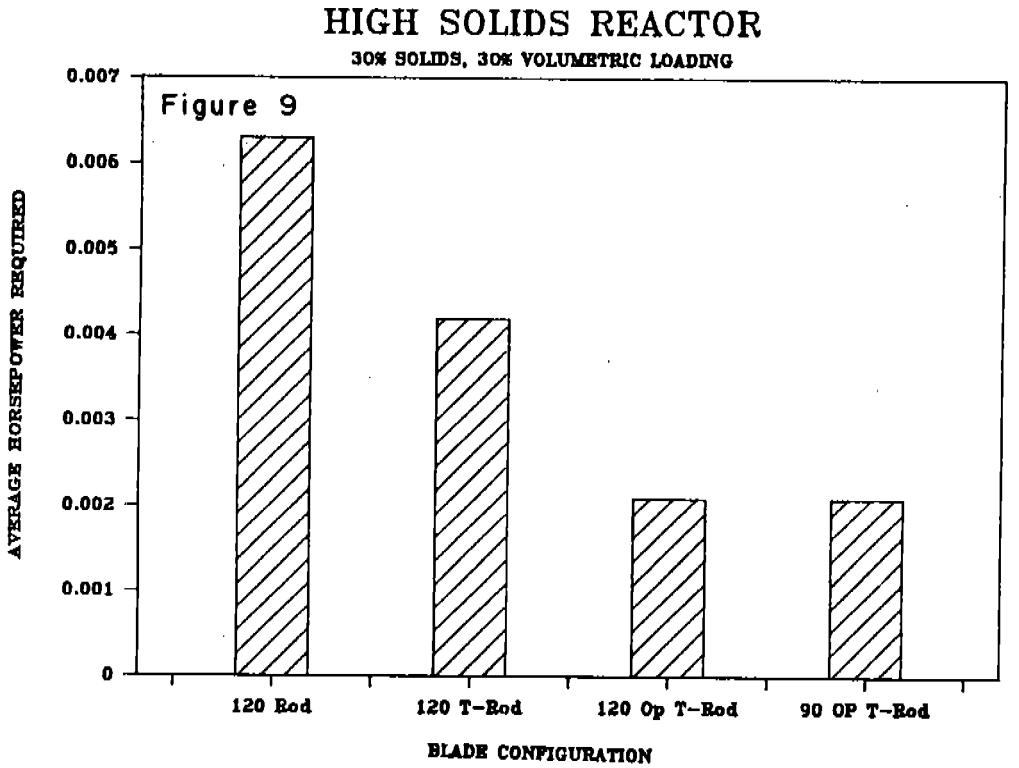
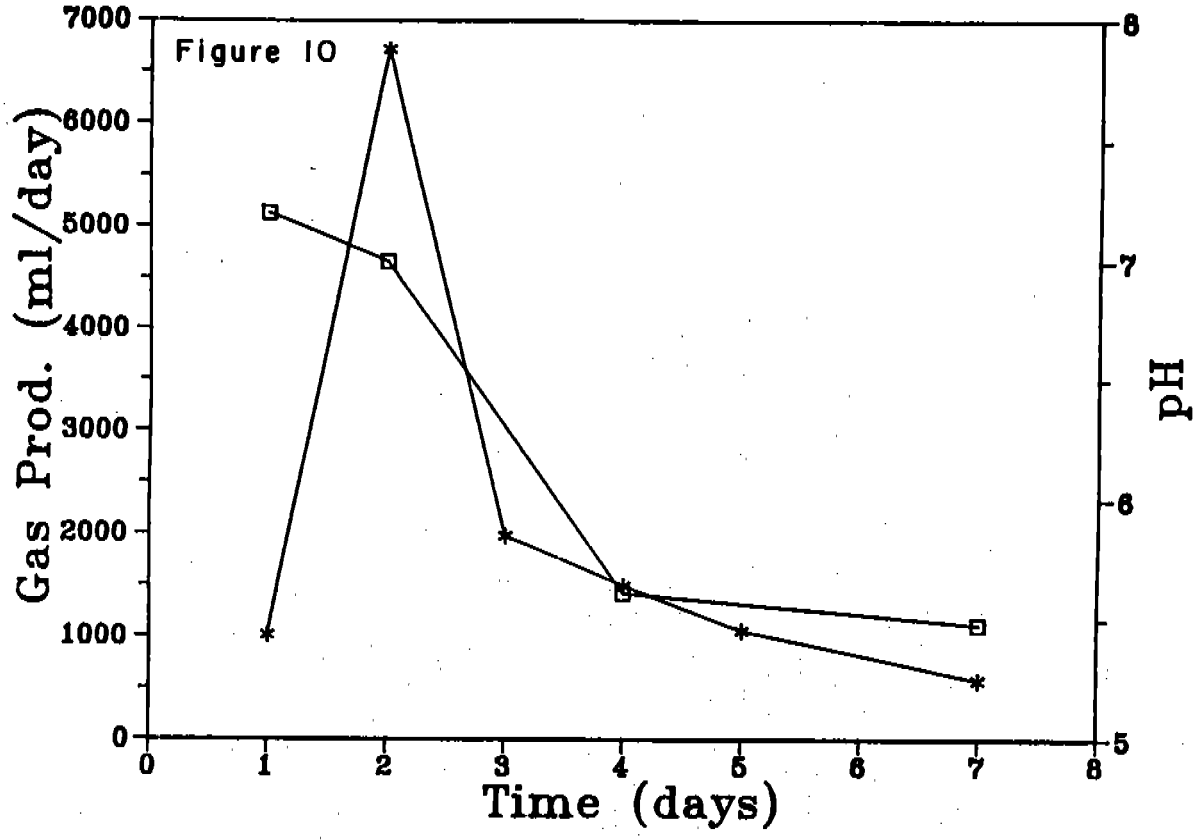


Figure 8 and 9. Average horsepower required for mixing with 180° rake, 120° rake, 120° rod, 120° teflon coated rod (T-Rod), 120° opposing teflon coated rods (Op T-Rod), and 90° opposing teflon coated rods.



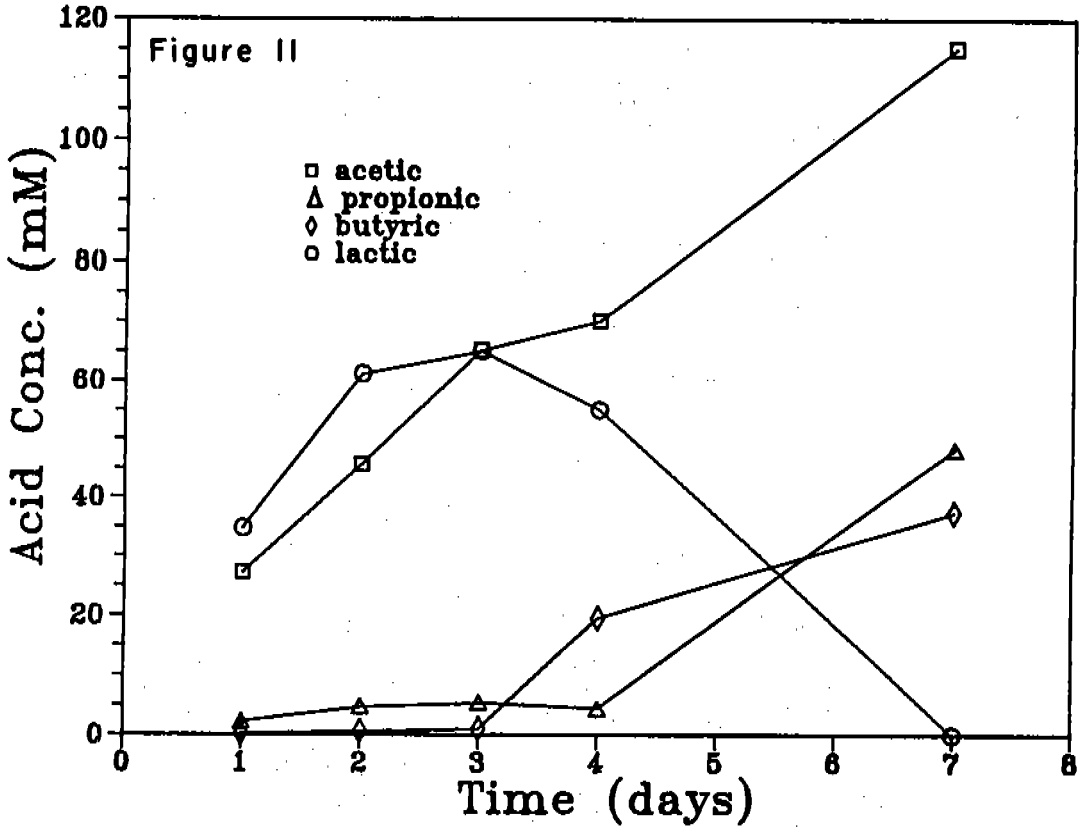
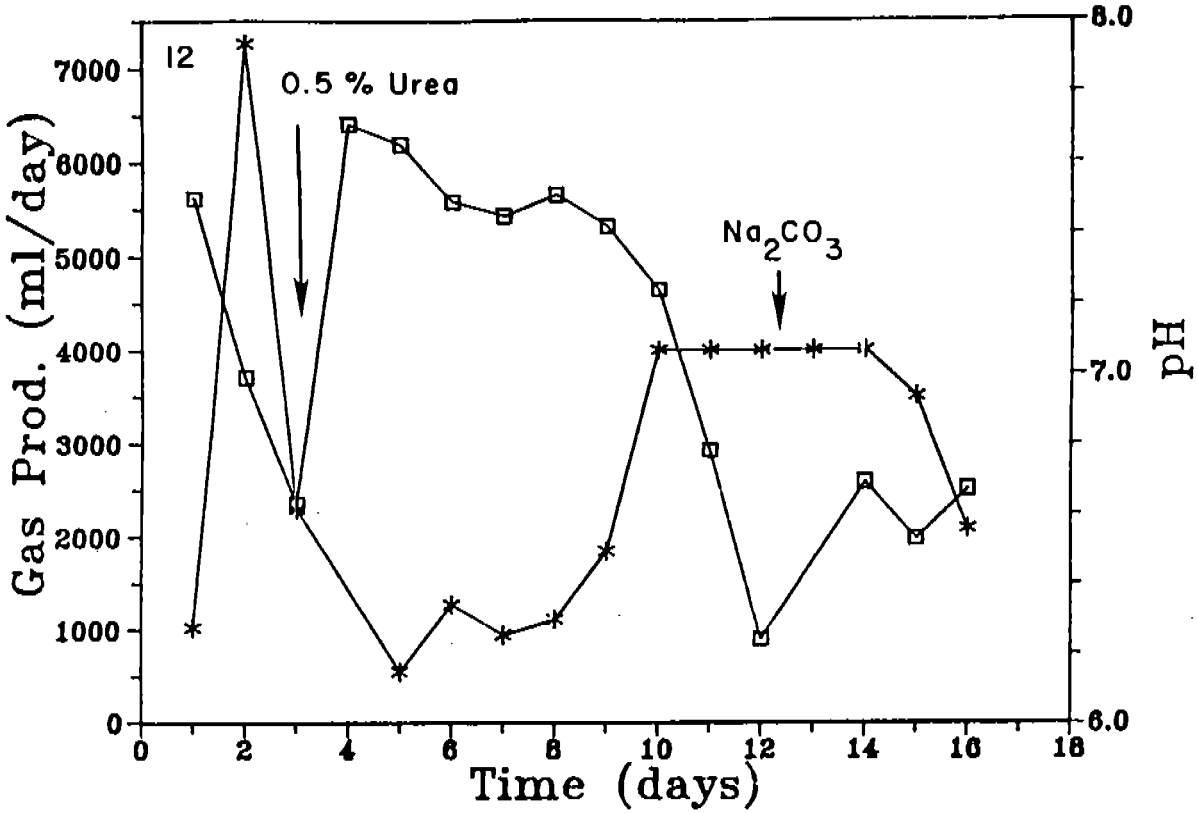


Figure 10 and 11. Total gas production, sludge pH and organic acid pools during operation of high solids run #2.



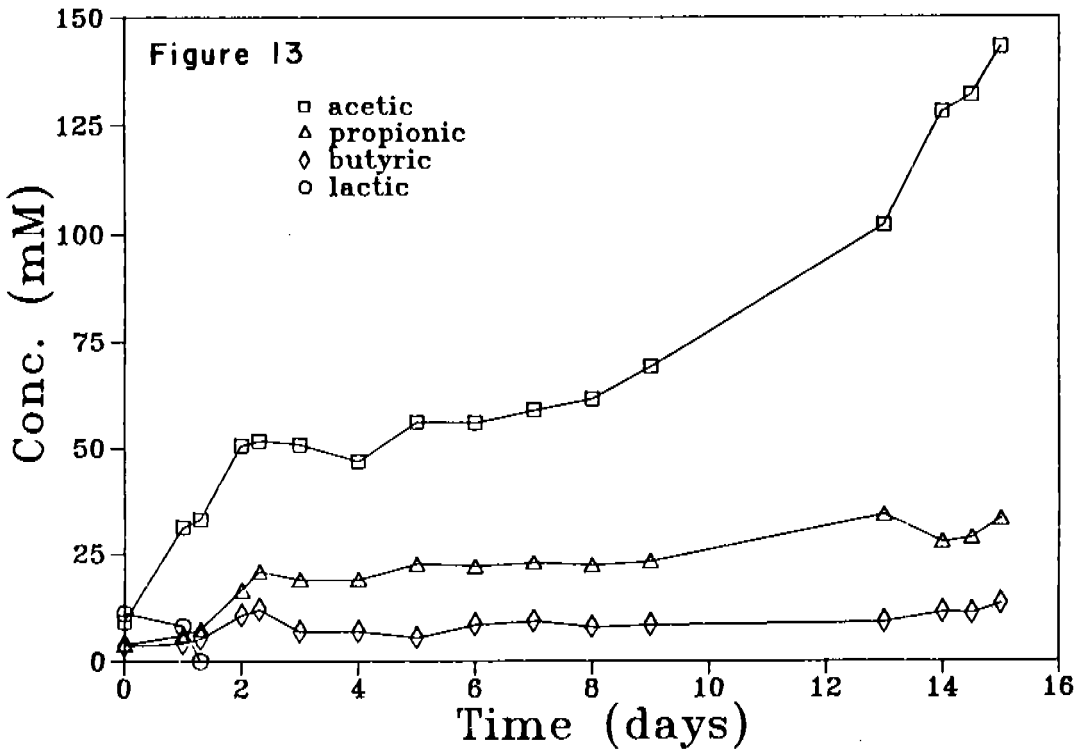


Figure 12 and 13. Total gas production, sludge pH, reactor additions, and organic acid pools during operation of high solids run #3.

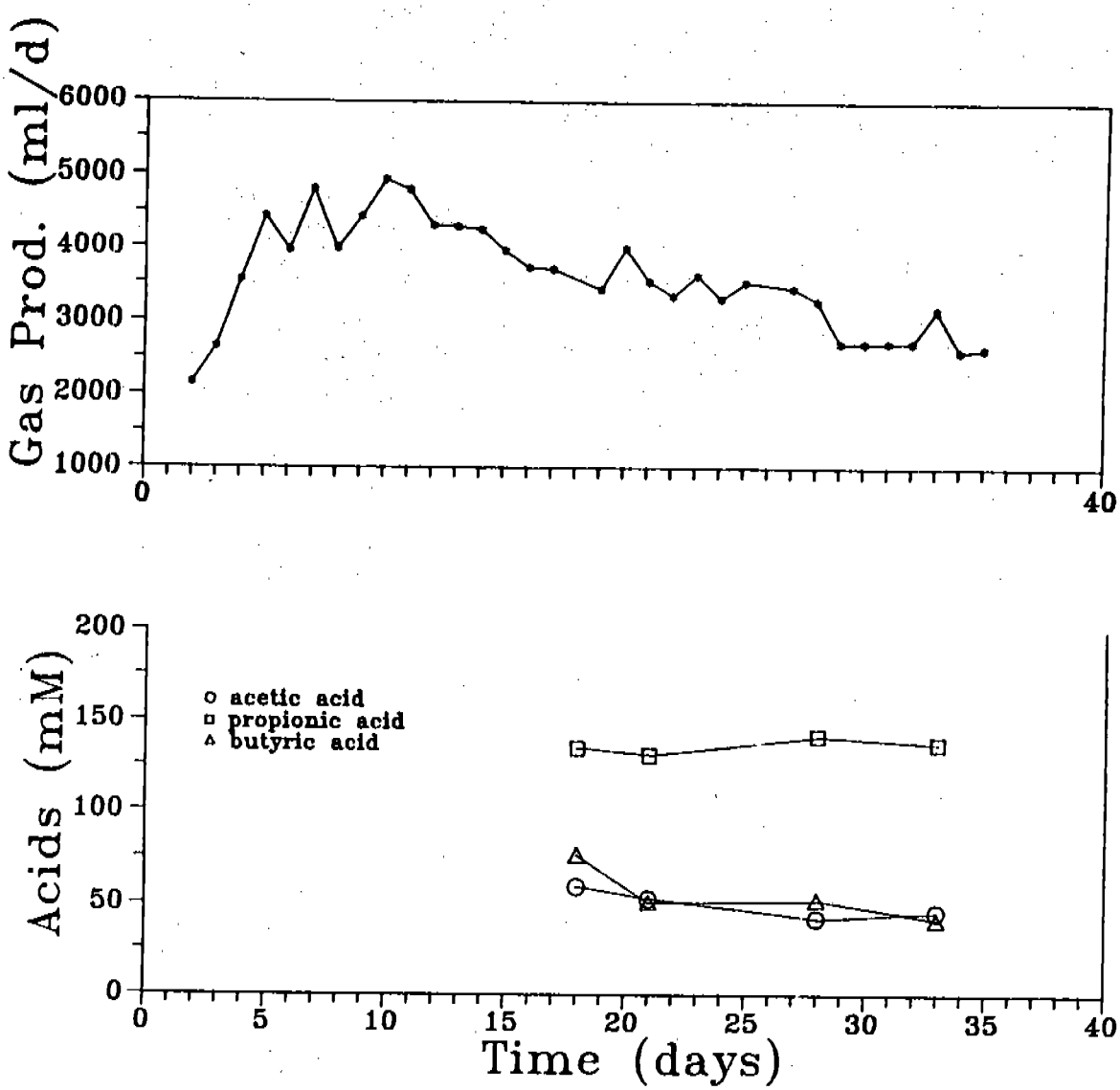


Figure 14. Total gas production and organic acid pools in the enhanced acetogen/methanogen fermenter.

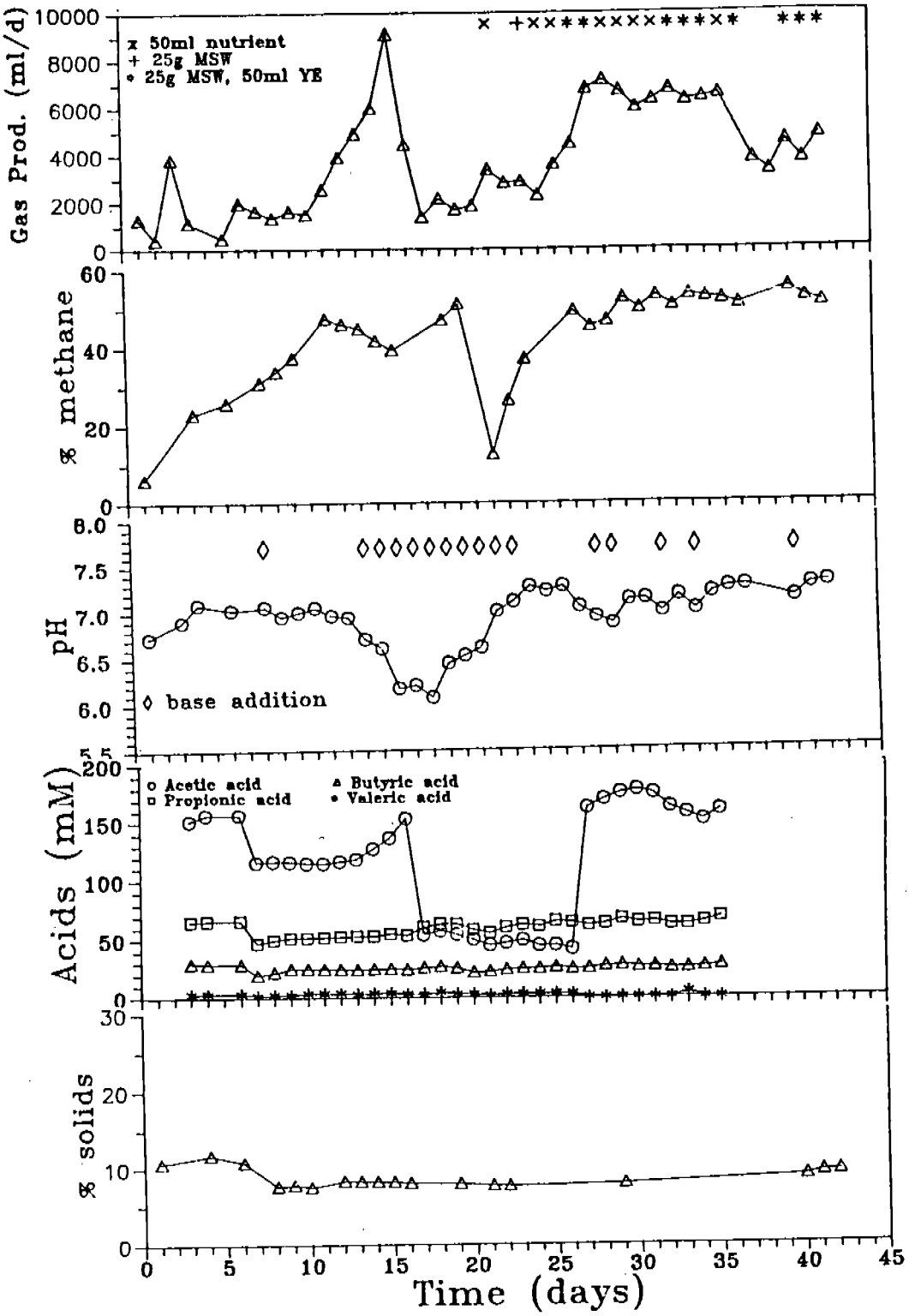


Figure 15. Fermentation performance of high solids run #4. The fermentation was initiated with addition of 3.6 Kg dry MSW. The MSW was mixed while outgassing overnight with oxygen-free nitrogen gas. A total of 7.4 liters of liquid was added of which 6.4 liters was digester sludge and 1 liter was nutrient solution. The nutrient solution contained 8g yeast extract, 50mM K_2HPO_4, 18g urea, and 120 ml of 10x trace mineral solution (Balch et al. 1979).

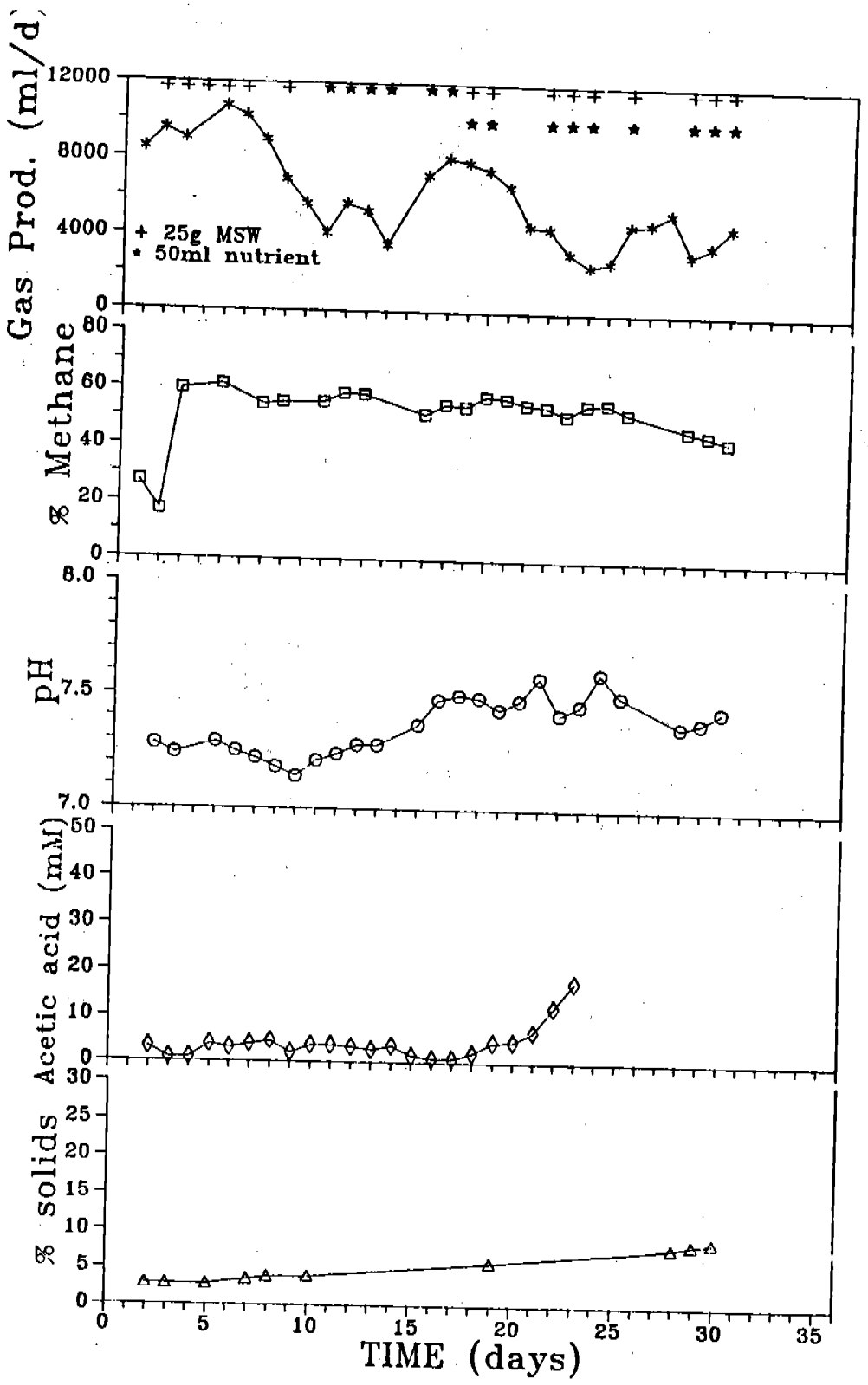


Figure 16. Current fermentation performance of high solids run #5. The fermentation was initiated with addition of 7 liters of digester sludge (approx. 3.5% solids). Solids are increased gradually by batch feeding of dry MSW (1/8 inch hammer milled).

BIOCONVERSION OF MSW AND RECOVERY OF ORGANIC ACIDS

Antonios A. Antonopoulos and Edward G. Wene
Energy and Environmental Systems Division
Argonne National Laboratory
Argonne, Illinois 60439

ABSTRACT

Research was conducted to determine the feasibility of bioprocessing municipal solid waste (MSW) to produce organic acids and recover the acids for hydrocarbon fuels production. This work focused on acid production from anaerobic digestion of a simulated MSW material, and densified refuse-derived-fuel MSW substrate, using 5-L continuously stirred and 12-L packed bed anaerobic digesters. Methane generation was inhibited by heat treatment, addition of a methanogen inhibitory compound, low pH, inoculum from a long-term acid adapted culture, and short retention times. Steady state operation was reached with acid concentrations of 15,000 to 18,000 mg/L. Nitrogen supplementations to the feedstock increased volatile acid concentrations to between 27,000 to 30,000 mg/L. Aerobic sewage sludge additions to feedstock increased peak levels of acid production to 22,000 mg/L. Acid extraction with trioctylphosphine oxide (TOPO) in heptane extracted more than 82% of the acid content in a single extraction of digester fluids with 15,000 mg/L total acids.

BIOCONVERSION OF MSW AND RECOVERY OF ORGANIC ACIDS

INTRODUCTION

An enormous tonnage of MSW is generated everyday that causes health, safety and environmental hazards. In the United States, for example, more than half a million tons of garbage (MSW) is generated per day. MSW is a candidate source for alternate fuels production. It is concentrated, must be disposed, contains valuable and reusable material, includes needed feedstock for liquid and gaseous fuels production, is an economic feedstock (tipping-fee required for disposal), and is available in large amounts daily throughout the year. It has been estimated that more than 65% of the MSW constituents are bioconvertible to produce fuels and other valuable compounds.

Under anaerobic conditions, MSW organic content is decomposed and digested by four microbial groups that interact in a complex ecosystem which is not favored by the presence of oxygen and other adverse factors and conditions [Barker, 1956; Zeikus, 1977; Bryant, 1979]. The four groups of bacteria involved in hydrolysis and finally methane production are: (a) hydrolytic-acidogenic (fermentative chemotrophic) bacteria, which hydrolyze carbohydrates and other polymers to organic acids, alcohols, CO_2 and H_2; (b) hydrogen-producing (obligate proton reducing) acetogenic bacteria, which metabolize the products of the previous group into acetate, H_2 and CO_2; (c) homoacetogenic bacteria, which metabolize H_2, CO_2 and formate to acetate; and (d) the methanogenic bacteria, which catabolize either acetate (acetophilic methanogens) or H_2 and CO_2 (hydrogenotrophic methanogens) to CH_4 and CO_2. The feedstock for anaerobic digestion is first converted to organic acids, and finally to CH_4 and CO_2. Therefore, if this conversion is terminated at the organic acid step, then these acids can be recovered and further utilized for hydrocarbon liquid fuels production.

There are several ways to discourage growth and activities of methanobacteria, and to encourage the proliferation of acid producing bacteria. The methanogens grow slowly and do not grow in the presence of oxygen and low pH, [Hungate, 1966; Mah, et. al., 1977; Zeikus, 1977; Bryant, 1979], therefore, controlled oxygenation, pH control, heat treatment, shortening the retention time, and addition of methanogenic inhibitory compounds reduce methanogenic activity. In addition, there are available methods for separation and recovery of the acids from the digestion fluid, and a few methods have been suggested for the conversion of organic acids to alkane hydrocarbons. Therefore, research supported by DOE at Argonne National Laboratory has focused on studying the feasibility of using MSW feedstock to produce short-chain organic acids, and to recover the acids and convert them to liquid hydrocarbon fuels. Efforts have focused on improving acid production from MSW feedstock, and the extraction of organic acids from digester fluids.

MATERIALS AND METHODS

Digesters. Two Virtis 43 Series Fermenters (Virtis Co., Gardiner, N.Y.12525) were used as anaerobic digesters. These digesters are continuously stirred tank reactors with a 5-L glass jar and stainless steel head assembly. They are supplied with agitation speed,

temperature and aeration controls. A separate module is included for pH readout and recording. In addition, a 12-L packed bed digester was constructed in our laboratory. It is made of 6-inch Pyrex pipe, 36 inches in height. The upper and lower sections are custom-made Pyrex fitted with sampling ports and hose fittings for effluent recycling and gas collection. A multihead peristaltic pump is used to recycle liquid from the bottom to the top of the reactor. The reactor is wrapped with heating tape, insulated and thermostatically controlled to maintain temperature control. The digesters are connected to 15-L liquid displacement gas collectors to monitor gas production.

Feedstock. The initial start-up of the anaerobic digestes utilized a medium that contained cellulose (Solka-Floc BW-40), mineral salts and nutrients. Thereafter, the feedstock was changed to a simulated MSW consisting of 77% paper products (60% newsprint, 15% cardboard, and 25% assorted paper), 20% food and garden wastes, and 3% textiles and rags. This feedstock was mixed and fed to the digesters in dry form or mixed with water. The digesters were also fed with densified-refuse-derived fuel (dRDF) pellets without binder from the Thief River Falls, Minnesota, MSW pelletizing facility. During one period of operation the feedstock was treated by soaking at room temperature for 24 hours in a mixture of 2% $Ca(OH)_2$ and Na_2CO_3.

Inoculum. The digesters were inoculated with anaerobic sewage sludge from the Wheaton Municipal Sanitary District (Wheaton, IL.). This inoculum has been maintained in this laboratory and been used to reinoculate the digesters. The inoculum has been periodically reseeded with fresh material from the Wheaton digester, extracts from active compost piles, and enriched sewage sludge incubated in this laboratory.

Digester Operation. The two Virtis digesters were initially fed with 2.5 L of simulated MSW (total solids concentration of 30 g/L) and seeded with 2.5 L inoculum. The feedstock and the inoculum was continuously mixed and allowed to stabilize with weekly feedings. The digester temperatures were maintained at 35°C with agitation rates of 150 rpm, and the pH was initially controlled to remain above 5.5. The solids loading rate was varied from 2.0 to 10.0 kg/VS/m^3-d to maintain the desired solids concentration. The solids loading rates were gradually increased and at the end of 100 days the total and volatile solids (TS and VS) were 30 g/L and 20 g/L, respectively. Each 5-L digester was maintained at a final volume of 4 liters.

The start-up of the digesters was followed by the development of acidogenic populations. The digester contents were heated to 80 C for 15 to 30 minutes to eliminate methanogenic bacteria and select for spore forming bacteria. Also, 2-bromoethane sulfonic acid (BESA, a compound inhibitory to methanogenic bacteria) was added to the digesters to a final concentration of 2×10^{-4}. BESA was added at twice weekly intervals to maintain the concentrations in the digesters at that level.

Methods of Analysis. Analyses were made to determine volatile acid concentration and composition, gas composition, alkalinity, TS and VS. The volatile acid concentration, alkalinity, TS, and VS were determined in accordance with Standard Methods for the Examination of Water and Wastewater. Gas composition and individual volatile acids were estimated by gas chromatography. Gas composition was determined using a Varian (Varian Instruments Division, Palo Alto, CA) 90P gas chromatograph with a thermal

conductivity detector and a Porapak Q column. Individual volatile acids were determined using a Gow Mac 750P (Gow Mac, Bridgewater, N. J.) gas chromatograph with a flame ionization detector and a 3.18 mm X 160 cm column packed with 100/120 mesh Chromosorb WAW with 15% SP 1220 and H_3PO_4 (Supelco, Bellefont, PA). The temperature program started with a column temperature of 100°C for 2 minutes then ramped 10 degrees per minute to 140°C. The injection port was held at 150°C and the detector at 200°C. Helium was used as the carrier gas at a flow rate of 35 mL per minute. Samples of digester fluid were prepared as follows: digester fluid was centrifuged at 4,000 X g for 10 minutes. Two-mL samples were acidified with two drops concentrated H_2SO_4, and two mL diethyl ether added. The two phases of the mixture were inverted at least 15 times and centrifuged if a stable emulsion developed. The gas chromatograph was calibrated with a standard solution containing 10 mM each of acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic, isocaproic, and heptanoic acid.

Acid Extraction. Trioctylphosphine oxide (TOPO) and reagent grade heptane (Sigma Chemical Co., St. Louis, MI) were used as received from the supplier, and acid solutions were prepared from reagent grade acids and distilled water. Acid solutions were contacted with the TOPO-heptane solution for more than one hour at room temperature in a 200-mL glass stoppered separatory funnel. The funnel was shaken and inverted a minimum of 200 times during this period and the phases were allowed to separate for at least one hour. Preliminary experiments showed that this contact time was sufficient for equilibrium. The final sample for testing was centrifuged at 5,000 X g for 5 minutes to insure complete phase separation after sampling. Acid concentrations were measured with a gas chromatograph, as above, without adding ether. The concentrations were determined by measuring the amount of acids remaining in the aqueous phase following extraction. They were not measured directly in the organic phase because the organic solvent peak interferred with several of the acid peaks on the chromatogram.

RESULTS AND DISCUSSION

Conventional anaerobic digesters operate at low solids concentrations, long retention times, and with low concentrations of organic acids. Two-stage digesters use high solids loading rates and short retention times in the first stage, which leads to increases in the organic acid concentration in this stage. Our studies have shown that an acid-phase culture operated on an eight-day retention time and a solids loading of 7-8% maintained an average volatile acid concentration near 10,000 mg/L. Methane continued to be produced under these preliminary operating conditions.

Several methods were tried during this period to reduce methane production. Periodic heat treatment of the digester was effective in eliminating methane production for short periods of time but also had an immediate effect on acid production. Acid production began to recover 3 to 5 days following heat treatment, while methane production returned about 7 to 10 days after treatment. There was an immediate elimination of methane production following the addition of BESA, however, volatile acid accumulation also decreased following this treatment.

A mild alkali pretreatment of the MSW cellulosic feedstock resulted in increased production and accumulation of volatile acids. The concentrations averaged 14,000 mg/L with alkali pretreated feedstock on an eight-day retention time. This treatment added alkalinity to the digester and pH control was not required to maintain the pH above 5.5. During this experiment the solids loading was increased to raise the total solids to near 7%. The volatile acid concentration at the beginning of this treatment was near 8,000 mg/L and rapidly increased to 17,000 mg/L. As this treatment continued the total concentration of volatile acids decreased from the peak of 17,000 and remained between 10,000 to 15,000 mg/L with minor fluctuations. The operation of the digester during this period demonstrated that it is possible to operate a digester at relatively high volatile acid concentrations on a continuous basis.

When the alkali petreatment was discontinued, within two retention times the volatile acids increased to 20,000 mg/L and the pH increased to near 7.0. This was followed by increased gas production and a decrease in the volatile acids to 12,000 mg/L. This rapid change in digester behavior is largely unexplained but may have been due to a decrease in the alkalinity after discontinuing the alkali pretreatment. Peak accumulation of volatile acids during this period suggested that high concentrations of acid could be produced under proper operating conditions. One factor which may have been limiting during this period was nitrogen. The high carbon/nitrogen ratio of the feedstock could be responsible for insufficient nitrogen.

When supplemental nitrogen in the form of ammonium sulfate and urea was added to the feedstock (total nitrogen supplemented to the feed was 0.1 g/L-day nitrogen), volatile acid production and accumulation increased. Average concentrations of volatile acids after several retention times increased to 16,000 mg/L. The addition of ammonium sulfate and urea was discontinued and the feedstock was supplemented with proteose peptone which supplied a protein equivalent of 0.1 g/L-day (nitrogen equivalent of 0.02g/L-day). The retention time of the digester during the addition of the proteose peptone was increased to 10 days. With this supplement added to the feedstock the volatile acid concentration began to increase from the beginning level of between 10,000 to 12,000 mg/L. After 5 retention times the concentration of volatile acids reached 30,000 mg/L. At the beginning of this period propionic acid accounted for nearly 10,000 mg/L of a total of 13,000 mg/L of volatile acids. As the supplement was added the proportion of acetic to propionic acid began to increase. The acetic acid accounted for nearly half of the total volatile acids of 30,000 mg/L, the propionic decreased to about 5,000 mg/L, and butyric acid increased to near 7,000 mg/L (Figure 1).

The increase in acid production with the supplementation with protein was more than could be accounted with the total conversion of the protein to volatile acids, that is, there appears to be an increase in cellulose hydrolysis with protein added. The microbial hydrolysis of celulose is the rate-limiting step in anaerobic digestion, therefore, tests were conducted to determine if acid production would continue in the absence of fresh substrate with continued additions of the protein supplement. Results showed that acid production decreased rapidly when fresh substrate was not added. This indicates there is a readily accessible portion of the MSW feedstock that is responsible for the rapid production of acids, while the remaining portion of the feedstock is less easily hydrolyzed by the bacteria.

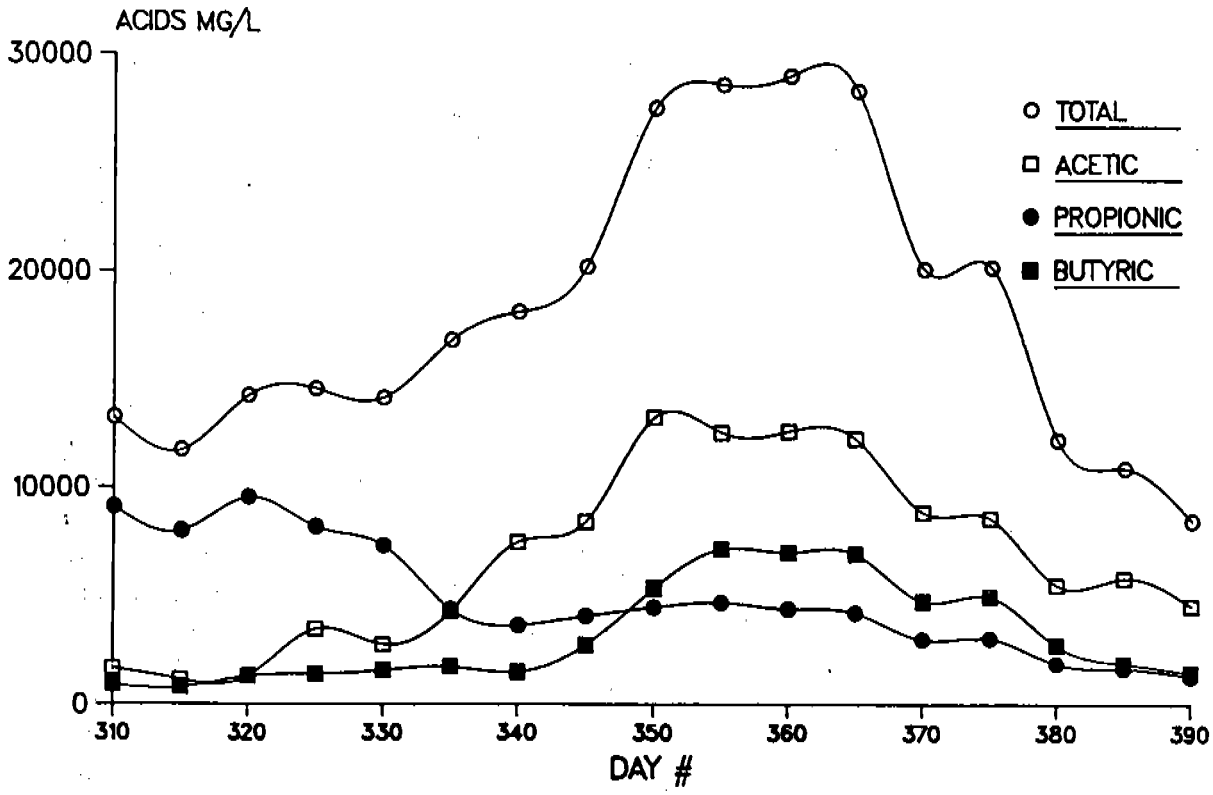


Fig. 1. Total volatile acid concentrations and the concentrations of individual acids in mg/L in a completely stirred 5-L digester.

The need for nitrogen supplementation of the feedstock was recognized and work was initiated to find an inexpensive source to replace the nitrogen and protein supplements. Aerobic sewage sludge was added to the feedstock to determine the effect on acid production. The sludge was added daily to the 5-L reactor as 10% by weight of the feedstock. The reactor was operating on a 10-12 day retention time with MSW feedstock which was a pelletized MSW (dRDF). An immediate increase in acid production and accumulation was observed in this reactor. Acid concentration increased from 12,000 to 22,000 mg/L within the first two retention times. Along with this increase in acid production there was also an increase in gas production. After several retention times the volatile acids dropped to 11,000 mg/L as a result of the gas production. It appears that with this feeding schedule and retention time that the methanogens must be controlled to increase acid production. Samples of an operating digester were extracted with 1 M TOPO in heptane. The digester had 15,000 mg/L total volatile acids. A single extraction procedure removed 82% of the volatile acids and there was no noticeable problem with phase separation due to suspended and dissolved material in the digester fluid.

Organic acid production and accumulation in the anaerobic digesters is limited because of the toxic effects of acids. Overall rates of production could be improved if the acids were extracted from the digester fluid in a continuous or batch process. Furthermore, one of the main objectives of this work is to recover the produced acids for making hydrocarbon fuels. Therefore, work focused also on the extraction and recovery of the acids. The extraction of acetic acid with TOPO in organic solvents has been studied by several investigators [Ricker,et. al, 1979; Shah, et. al., 1981; Golob, et.al., 1981.], but information on the effectiveness of the extraction procedure on acids of longer chain length than acetic is limited.

Initial experiments (Table 1) showed the effect of different concentrations of TOPO in heptane and the effect of heptane alone on extracting organic acids from aqueous solutions.

Table 1. Effect of TOPO Concentration in Heptane on Extraction of Organic Acids from Aqueous Solutions.

TOPO Concentration in Heptane	PPM Acids Extracted from 1,000 PPM of Each Acid						
	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Caproic
1.00 M	870	880	1000	1000	1000	1000	1000
0.75 M	760	950	1000	1000	1000	1000	1000
0.50 M	560	910	980	980	1000	1000	1000
0.25 M	330	920	1000	1000	1000	1000	1000
0.10 M	150	710	910	1000	1000	1000	1000
0.01 M	70	240	450	360	680	640	1000
0.00 M	60	150	290	250	530	490	850

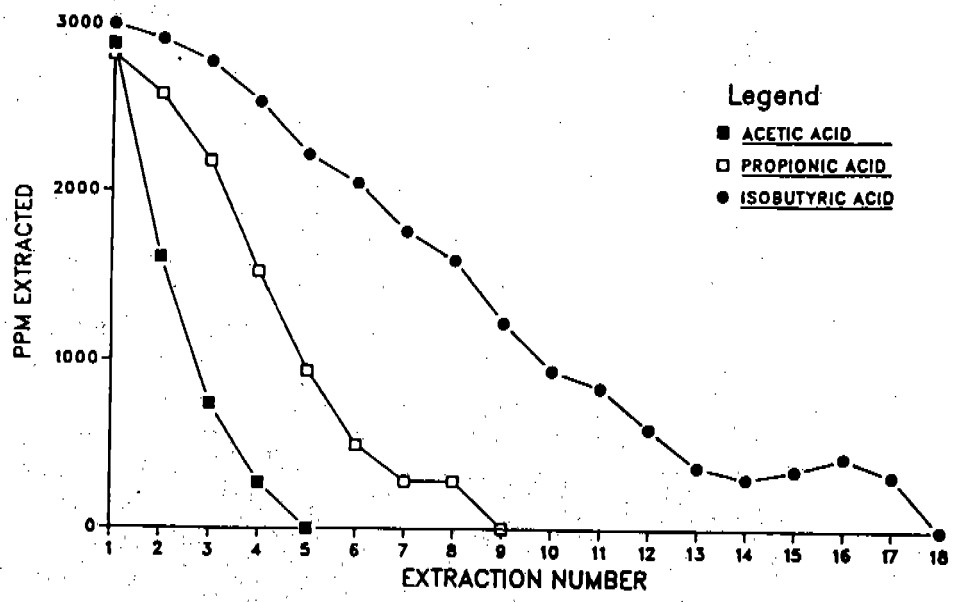


Fig. 2. Effect of repeated extractions using 1M TOPO in heptane on aqueous solutions containing 3,000 ppm of each acid.

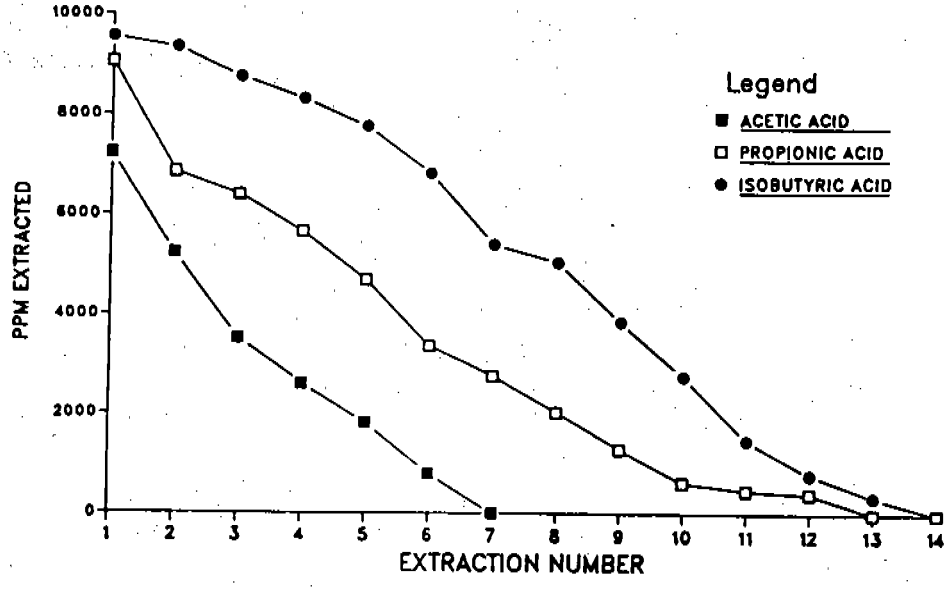


Fig. 3. Effect of repeated extractions using 1M TOPO in heptane on aqueous solutions containing 10,000 ppm of each acid.

The data shows that acetic acid is more resistant to extraction with TOPO and heptane than the longer chain acids. The next step was to determine the total amount of acids that could be extracted with TOPO and heptane. Figures 2 and 3 show the results of repeated extraction of solutions of acetic, propionic, and isobutyric acid at two different concentrations. In these tests, a solution of either 3,000 or 10,000 ppm of each acid was repeatedly extracted with the same solution of 1M TOPO in heptane. The organic phase was reused for each subsequent extraction and the aqueous phase was renewed for each extraction.

A 12-L packed bed reactor has been operated with MSW feedstock with mixed results. Rapid increases in volatile acid concentration have been produced due to the high solids content of the reactor but operational problems have prevented long term stability. Most of the difficulties have been due to packing of the substrate within the reactor.

Preliminary serum bottle tests have showed no toxic effect of the extracting fluid on acid and gas production. Serum bottle contents have been extracted up to five times without effect. Tests are presently underway on larger reactors to determine optimum extraction procedures to maximize acid production.

Future work needs to be conducted to: (a) achieve stable operating conditions in digesters utilizing MSW feedstock to produce organic acids; (b) maximize acid production from MSW bioconversion; (c) identify the most effective method for organic acid recovery and extraction; and (d) determine the most feasible and effective method for converting organic acids to hydrocarbon fuels.

ACKNOWLEDGEMENTS

This research is supported by the Department of Energy, Assistant Secretary of Conservation and Renewable Energy, under Contract W-31-109-ENG-38.

REFERENCES CITED

Barker, H. A. 1956. "Biological Formation of Methane," In: H. A. Barker (ed.), Bacterial Fermentations, John Wiley & Sons Inc., New York.

Bryant, M. P. 1979. "Microbial Methane Production - Theoretical Aspects," J. Animal Sci. 48: 193-201.

Golob, J., Orlic, V., and Zadnih, B. 1981. "Extraction of Acetic Acid from Dilute Aqueous Solutions with Trioctylphosphine Oxide," Ind. Eng. Chem. Process. Res. Sev. 20: 433-55.

Hungate, R. E. 1966. The Rumen and Its Microbes. Academic Press, New York.

Mah, A. R. Ward, D. M., Baresi, L., and Glass, T. L. 1977. "Biogenesis of Methane," Ann. Rev. Microbiol. 31:309-41.

Rucker, N. L., Michaels, J. N., and King, C. J. 1979. "Solvent Properties of Organic Bases for Extraction of Acetic Acid from Water," J. Separ. Proc. Technol. 1: 36-41.

Shah, D. J., and Tiwari, K., 1981. "Recovery of Acetic Acid from Dilute Aqueous Streams Using Liquid-Liquid Extraction with Tri-n-butyl Phosphate as Solvent," J. Separ. Proc. Technol. 2: 1-6.

Standard Methods for the Examination of Water and Wastewater. 14th ed. 1979. American Public Health Association, Washington, D. C.

Zeikus, J. G. 1977. "The Biology of Methanogenic Bacteria, " Bacteriol. Rev. 41: 514-41.

THE MICROAEROPHILIC DIGESTION OF PROCESSED MUNICIPAL SOLID WASTE FOR THE PRODUCTION OF ORGANIC ACIDS

Christopher J. Rivard, Todd B. Vinzant, William S. Adney, and Karel Grohmann
Biotechnology Research Branch
Solar Energy Research Institute
Golden, Colorado 80401

ABSTRACT

The effectiveness of microaerophilic digestion of processed municipal solid waste was compared to strictly aerobic and strictly anaerobic digestions with the same feedstock. The processed MSW was apparently poor in nutritional value needed to maintain a robust bacterial population, and research was conducted to identify the nutrient requirements of both the aerobic and anaerobic microbial populations for optimum digestion rates. Initial microaerophilic digester operation is reported.

THE MICROAEROPHILIC DIGESTION OF PROCESSED MUNICIPAL SOLID WASTE FOR THE PRODUCTION OF ORGANIC ACIDS

INTRODUCTION

A limiting step in the conversion of lignocellulosic materials in MSW has previously been shown to be the hydrolysis of cellulose and hemicellulose (Boone 1982). This is especially true since novel anaerobic digesters (e.g. packed bed anaerobic filters) can be operated on sugars or organic acids at a rate which is at least an order of magnitude faster than digesters fed raw feedstocks such as MSW (Barry 1982, Rivard 1985, Suidan 1983, Young 1969). As a result, the current popular concept in anaerobic digestion involves two stage fermentation. In the first stage (usually a percolating bed), MSW is hydrolyzed, and the resulting soluble compounds are partially converted to short chain fatty acids, hydrogen and carbon dioxide. The product from the first stage is fed to a methane production stage (usually a packed bed digester) where the soluble compounds are converted to the end-products methane and carbon dioxide. Part of the liquid effluent is usually recycled back to the first stage for nutrient value.

A two stage system has inherent advantages over single stage digesters. The numbers of degrees of freedom for optimization of the overall system is higher, the reactors can be optimized for the incoming streams (appropriate conditions selected for solids in the first stage and liquids in the second), and the recycle of liquid streams improves the water management, which is always a consideration in fermentations. Typically, the first step is conducted anaerobically, but the low energy yield which anaerobes obtain by fermentation results in slow rates of hydrolysis (Boone 1982, Bryant 1979). However, if the first stage is operated under microaerophilic conditions, improved energy conversion may lead to higher microbial biomass and consequently improved production of hydrolytic enzymes (cellulases and hemicellulases). Evidence supporting this concept comes from research results on composting. Composting of MSW performed under aerobic or microaerophilic conditions takes several days whereas anaerobic composting takes months. Recent results with controlled landfills also indicate that landfills which are only partially flooded by groundwater and partially oxygenated in the top layers seem to produce gas earlier and faster than completely flooded (anaerobic) ones.

To determine the effectiveness of microaerophilic digestion of MSW, the rate of conversion must be compared to strictly aerobic and strictly anaerobic digestion rates at optimum conditions for each. In the determination of the maximum rates of aerobic digestion of MSW, the nutritional components required to support the higher microbial biomass production must be examined. This is especially true for processed municipal solid waste in which the food and yard wastes have been removed for composting operation and to reduce the moisture content of the MSW for pelletizing and storage.

MATERIALS AND METHODS

Anaerobic and Aerobic Digesters

Four anaerobic digesters with 3.5 liter working volumes were constructed and operated as previously described (Henson 1986, Rivard 1987) with the exception of heat tape (1 inch x 6 ft, Briskheat) and a temperature controller (model 601, Omega) used to maintain the 37°C constant temperature. The three aerobic reactors were 2 liter Applikon fermenters (1.2 liter working volume) complete with motor speed controller, pH control (model 704, Horizon Ecology) and temperature control at 37°C via temperature controlled circulating water bath (Model 8000, Fisher Scientific). Both aerobic and anaerobic reactors were batch fed daily. The feedstock was knife milled (1 mm rejection screen, Wiley Mill) processed and densified municipal solid waste obtained from Thief River Falls, MN. (Future Fuels Inc.). Nutrient addition concentrations are as described and were yeast extract (Difco), trace mineral addition (Balch 1979), all other chemical components were research grade reagents. Effluent was removed on a daily basis and stored at 4°C until analysis were performed.

Redox Digester

The redox digester consisted of a converted 2-liter Multigen fermenter (New Brunswick, 1.5-liter working volume). The temperature was controlled using heat-tape to the outside of the glass vessel and an Omega temperature controller (model 601) with K-type thermocouple. The pH was maintained using a pH controller (Markson, model 6300) and a gel-filled pH probe. The redox was controlled using a pH/ORP controller (Horizon Ecology Co., model 5997) and an Ingold redox probe (Pt-4865-K9). Redox probes were changed and cleaned weekly. The ORP controller operated a 110 volt solenoid valve which sparged air to the reactor. The "house" air was first humidified using a sparging bottle filled with distilled water to reduce the chance of water loss in the reactor from aeration.

Sludge Analysis

Levels of volatile organic acids (C1-C5 iso and normal acids) were determined by gas-liquid chromatography (GLC). A model 5840A gas chromatograph (Hewlett-Packard, Palo Alto, California), equipped with a flame ionization detector, a model 7672A autosampler, and a model 5840A integrator (all from Hewlett-Packard), was used. The glass column (6 ft x 2mm) was packed with Supelco 60/80 Carbopack C/0.3% Carbowax 20M/ 0.1% H3PO4. The injection port temperature and detector were maintained at 190°C. The oven temperature was maintained at 120°C. Nitrogen at a flow rate of 50ml/min was used as the carrier gas. The experimental samples were calibrated against a quantitative standard (Supelco) with each run. Experimental samples were prepared for analysis by centrifugation to remove suspended solids in 1.5ml micro-centrifuge tubes in a microcentrifuge (Eppendorf) at room temperature for 15 minutes. The clarified samples were acidified by addition (1:1) with 1 % v/v formic acid. Finally, the samples are loaded into autosampler vials, crimp caps attached and loaded into the autosampler for analysis.

Analysis of non-volatile organic acids in digester sludge effluent was accomplished by high performance liquid chromatography (HPLC). Experimental samples are clarified by centrifugation as described above with final removal of particulates by passage through a 0.2 micron Acrodisc disposable syringe filter (Gelman #4418). The samples were made 0.01 N with sulfuric acid and loaded into autosampler vials for analysis. The HPLC system consisted of a Beckman model 501 autosampler, Beckman model 110A pump, temperature controlled column (Eldex), Variable Wavelength Detector (detection at 210nm, Waters and Assoc.), and integrating recorder (Hewlett Packard, 3390). The column used was a Bio-Rad HPX-86 organic acids packed column. The column temperature was controlled at 45°C. The eluent was 0.01 N sulfuric acid in distilled water which was filtered (0.2 micron) and degassed before use. The flow rate of eluent was maintained at 0.5 ml/min. Samples were analyzed in conjunction with high purity standard mixtures of organic acids (Supelco). Analysis by HPLC identified both non-volatile and volatile and therefore served as a backup to the gas-liquid chromatography analysis.

Solids analysis of digester effluents was conducted using 1.0 gram aluminum weighing tins and dispensing approximately 20-30 ml of sludge in duplicate. The sludge samples are dried for 48 hours at 45-50°C, cooled to room temperature in desiccators and weighed on a top loading balance (Sartorious model 1264MP).

Analysis of digestion rates and extent was assessed by determination of acid detergent fiber. This analysis results in values for acid detergent solubles (microbes, fats, protein), cellulose, lignin, and ash. The analysis protocol is as previously described (Rivard 1987, Goering 1970).

Gas Analysis

Production of biogas from anaerobic reactors was monitored on a daily basis (in conjunction with batch feeding) using calibrated water displacement reservoirs.

Biogas produced in the various digesters was analyzed for methane and nitrogen composition by gas chromatography. A Gow-Mac (model 550) gas chromatograph equipped with a thermal conductivity detector and integrating recorder was used. The injection port, oven, and detector were maintained at 100, 90, and 110°C respectively. Helium served as carrier gas. The chromatograph was calibrated with high purity gas standards (Matheson).

Microbiological Analysis

Observation of live microbial samples from digesters were conducted using wet-mount slide preparations with a Nikon Labophot microscope equipped with phase contrast, epi-fluorescent illumination, and 1000x power. Methane producing bacteria were detected by their unique auto-fluorescence upon excitation by the epi-fluorescent light source (narrow band with centered at 420 nm).

Elemental Analysis

Analysis of the important micronutrients, iron, nickel, molybdenum, aluminum, cobalt, manganese, etc., was accomplished using a arc-plasma spectrometer. The methods used have been previously described (Nygaard 1985, Tzavaras 1984, U.S. Environmental Protection Agency 1982). High purity elemental standards were prepared and used to calibrate the equipment.

Nitrogen (Ammonia) Detection

The analysis of excess free nitrogen in the form of ammonia in digester effluent was determined with the use of an ammonia gas sensing probe (orion electrodes). The millivolt signal was correlated using a standard curve to millimolar ammonia present as previously described (Rivard 1987). Ammonium chloride (reagent grade) standards were used to calibrate the probe and meter.

Phosphate, Sulfate, Nitrate, and Nitrite Analysis

The analysis of the various ion levels in digester effluent were determined using high pressure liquid chromatography. A Hewlett Packard model 1090 HPLC equipped with autosampler, ion conductivity detector (Wescan), and integrating recorder (Hewlett Packard, model 3392) was used. A Hewlett Packard model 85B computer and model 9121 dual disk drive, continuously monitored and controlled the HPLC operation. For separation, a Bio-rad anion exchange column (269-001) was maintained at 22°C. The eluent was 5 mM p-hydroxybenzoic acid (PHBA) adjusted to pH 8.5 in distilled water. The eluent was filtered through 0.45 micron filters and degassed before use. Calibration of the HPLC was made using reagent grade K_2HPO_4, Na_2SO_4 and $NaNO_3$. Digester effluent samples were prepared for analysis as described above for non-volatile organic acid analysis.

RESULTS

The Municipal Solid Waste (MSW) used in this research was that obtained from the processing plant in Thief River Falls, MN. The MSW was obtained in a large amount (several tons) and is in a dry, pelletized, stable form suitable for long term research. The composition of the MSW was determined as 26% acid detergent solubles, 52% cellulose, 20% lignin/plastics, and 2% ash by dry weight. Initial studies using a 1 mm knife milled form of the MSW resulted in an unstable anaerobic fermentation when the MSW was mixed with distilled water for feeding. Therefore, analysis of nutrient levels was undertaken. In Table 1, the available nitrogen was determined over a period of months during which various changes were made to the composition of the liquid portion of the feed. With addition of water to the MSW, the level of free ammonia was limiting in the anaerobic digesters as determined by the ammonia levels present in the digester effluent (<0.1 mM). With the addition of the standard supplement of 8 grams per liter of yeast extract, which served as a complex nutrient as well as nitrogen source, the nitrogen levels in the digester effluent were in the 30-40 mM range indicating the nitrogen demand was met. The aerobic digesters required the addition of ammonium chloride as well as

yeast extract to meet the nitrogen requirements. It is apparent that the maximum addition rate of 150mM ammonium chloride is not enough to meet the demand for nitrogen in the shortest retention time aerobic reactor.

Table 1. Effluent Ammonia Levels in Aerobic and Anaerobic Reactors.

Reactor	Operation	Retention time (days)	No Supplement	Standard Supplement	Addition of NH_4Cl 50mM	100mM	150mM
A	anaerobic	14	<0.1mM	34mM			
E		20	<0.1mM	37mM			
B		30	<0.1mM	38mM			
C		30	<0.1mM	-----			
Delta	aerobic	12	<0.1mM	<0.1mM	<0.1mM	<0.1mM	<0.1mM
Omega		20	<0.1mM	<0.1mM	<0.1mM	<0.1mM	2.4mM
Beta		30	<0.1mM	<0.1mM	<0.1mM	1.5mM	23mM

Determination of phosphate levels in the effluent of the aerobic and anaerobic reactors indicated that with addition of water or the standard yeast extract supplement, the level of phosphate was below the limit of detection by HPLC (<0.1mM). Therefore, the addition of potassium phosphate to the standard supplement used in feeding the reactor was initiated and in Table 2 resulted in sufficient levels of phosphate in the reactor effluent from both anaerobic and aerobic reactors that the requirement had been met, as evidenced by an excess of at least 20 mM phosphate.

Table 2. Effluent Phosphate levels in Aerobic and Anaerobic Reactors.

Reactor	Operation	Retention time (days)	No Supplement	Addition of K_2HPO_4 50 mM
A	anaerobic	14	<0.1mM	48mM
E		20	<0.1mM	40mM
B		30	<0.1mM	37mM
C		30	<0.1mM	-----
Delta	aerobic	12	<0.1mM	46mM
Omega		20	<0.1mM	38mM
Beta		30	<0.1mM	27mM

The effects on addition of a complex nutrient to supply necessary micronutrients such as vitamins and cofactors was investigated using yeast extract. The effects of various levels of addition of yeast extract in the feed was

compared to the resulting extent of conversion of the cellulose fraction of the feedstock (Table 3). In the anaerobic reactors the addition of 4 grams per liter of yeast extract in the feed resulted in significant increases in conversion of cellulose whereas the two fold increase in yeast extract to 8 grams per liter did not significantly alter the conversion. The aerobic digesters indicated a similar pattern with lower overall rates of breakdown of the cellulose portion of the MSW feedstock.

Table 3. Effects of Complex Nutrient Additions on Digestion Rates.

Reactor	Operation	Retention time (days)	---% Conversion of Cellulose in MSW--- No Supplement	yeast Extract Addition 4g/l	8g/l
A	anaerobic	14	---	---	78
E		20	---	77	80
B		30	35	79	83
C		30	35	---	---
Delta	aerobic	12	---	54	59
Omega		20	---	69	79
Beta		30	---	83	88

The levels of major elemental nutrients such as iron, nickel, cobalt, molybdenum, and manganous were determined by plasma-arc spectrometry. All elements with the exception of iron were determined to be present at levels below the detection limit (<0.1 ppm), when either the feed was supplemented with distilled water or the standard yeast extract addition. The supplementation of a 10X mineral solution (Balch 1979) resulted in sufficient levels of all elements (1-8 ppm) in the anaerobic digesters. In the aerobic reactors, only the levels of cobalt, nickel, and molybdenum were below 1 ppm. A 10X mineral solution with two fold higher additions of cobalt, nickel, and molybdenum was used in the aerobic reactors and resulted in improved levels (2-6 ppm) of these elements after two months. The levels of various ions was analyzed by ion chromatography (HPLC) and indicated no detectable levels of sulfate, nitrate, and nitrite in either aerobic or anaerobic reactor effluents.

Current fermentation parameters for the anaerobic digesters are shown in Table 4. The fermentations are stable, not requiring pH adjustment with the exception of digester "C" which is not receiving any supplements to the MSW feed. Substantial breakdown of the cellulose portion, which constitutes over 50% of the dry weight of the MSW feedstock, occurred in all reactors with the exception of digester "C" in which no nutrients were added.

Table 4. Anaerobic Digester Parameters.*

Reactor	RT (days)	pH	total biogas (ml/day)	biogas prod. (vol/vol/day)	% methane	% cellulose conversion	total acids**
A	14	7.20	6015	1.72	54.3	80	2.56
E	20	7.25	4186	1.2	61.6	81	1.27
B	30	7.25	2748	0.79	62.0	85	1.33
C	30	7.04***	521	0.15	69.5	35	5.76

* Data represents averages from the most recent 2 month period with the standard supplement added to the MSW including 8g/l yeast extract, 50 mM potassium hydrogen phosphate, and 10X mineral solution.

** Total acids include C2-C5 volatile acids and are expressed in millimolar concentration.

*** Digester requires addition of base on a regular basis to maintain pH.

Data from initial studies conducted under controlled oxidation/reduction conditions for the conversion of the MSW feedstock to organic acids is shown in Figure 1. Several important parameters have dramatically affected the effectiveness of the conversion process. The initial redox, pH and temperature conditions (-350 mv, 7.2, 37°C) under which the reactor was maintained resulted in initial production of acids but was soon followed by increasing populations of methanogenic microorganisms (auto-fluorescing cells under 420 nm microscopic illumination) in the microflora and decreasing levels of acids. Attempts to control the redox at higher levels (-200 to -300 mv) resulted in malfunctions in the controller due to the extended period of time required to raise the redox by gassing with air. The malfunctioning controller decreased gassing and lowered the redox, (-350 to -450 mv), resulting in increased numbers of methanogenic bacteria and lower acid levels.

DISCUSSION

The poor initial performance of the anaerobic digesters on the MSW using distilled water indicated a nutritional limitation. Analysis of effluents from the anaerobic and aerobic digesters indicated limiting concentrations of nitrogen, phosphate, elements, and most likely complex organics supplied by the addition of yeast extract. Over a ten month period the levels of each nutrient required to meet the demand for both anaerobic and aerobic digestions of MSW was determined. Optimization of anaerobic fermentation conditions resulted in stable operation and substantial degradation of the cellulose portion of the MSW feedstock. However, strictly aerobic digestion has not been optimized and one or more nutrient limitations still exist, especially in the low retention time reactor.

The redox digester which results in microaerophilic operation has encountered minor difficulties in controlling equipment. The redox parameter

has not been effectively controlled and as a result increasing populations of auto-fluorescing methanogenic bacteria are present in the digester microflora. The methanogenic cells observed under epi-fluorescence microscopy are of similar morphology and are rods occurring singly. The microbial population changes have reduced the organic acid pools in the effluent of the reactor to excessively low levels. It is expected that by variation in redox, pH, and possibly temperature, the microbial consortium of the reactor may be tailored to the production of organic acids without substantial utilization of acids produced.

FURTHER RESEARCH

Nutritional requirements for anaerobic digestion have been identified and good conversion has been demonstrated in the 14 day retention time reactor, Therefore, the shortest possible retention time will be investigated. The 20 and 30 day retention time anaerobic reactors will be shortened to 5 and 10 day retention time, and performance monitored along with effluent nutrient levels.

The nutritional requirements of the aerobic reactors has not been fully identified and will be expanded to include sulfur levels. As in the anaerobic reactors, when the nutritional requirements have been met and good conversion rates for the cellulose portion of the MSW have been demonstrated, the two longest retention time reactors will be changed to 4 and 8 day RT to identify the maximum rate of conversion under aerobic operation.

Two new reactors will be put on-line in addition to the current redox reactor to increase the amount of information gathered on the effects of variation in operational parameters under controlled redox fermentation of MSW. The ORP controlling problem will be alleviated by use of a Markson model 6300 pH/ORP controller which does not rely on a time delay alarm for the high and low limit operation. The presence of methanogenic bacteria, primarily in rod forms, during periodic gassing of the sludge with air is surprising, and the microbes will be briefly isolated and identified.

REFERENCES

Balch, W.E., Fox, G.E., Magrum, L.J., Woese, C.R., and Wolfe, R.S. 1979. "Methanogens: Reevaluation of a Unique Biological Group," Microbiol. Rev. 43:260-296.

Barry, M., and Colleran, E. 1982. "Anaerobic Digestion of Silage Effluent Using an Upflow Fixed Bed Reactor," Agric. Wastes 4:231-240.

Boone, D.R., 1982. "Terminal Reactions in the Anaerobic Digestion of Animal Waste," Appl. Environ. Microbiol. 43:57-64.

Bryant, M.P., 1979. "Microbial Methane Production--Theoretical Aspects," J. of Animal Science 48:193-201.

Goering, H.K., and VanSoest, P.J. 1970. "Forest Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications)," U.S. Dept. of Agriculture Handbook #379.

Henson, J.M., Bordeaux, F.M., Rivard, C.J., and Smith, P.H. 1986. "Quantitative Influences of Butyrate or Propionate on Thermophilic Production of Methane from Biomass," Appl. Environ. Microbiol. 51:288-292.

Nygaard, D.D., Chase, D.S., and Leighty, D.A. 1985. "Analysis of Wastewaters by ICP Spectrometry," Application Note #177.

Rivard, C.J., Himmel, M.E., and Grohmann, K. 1985. "Utilization of Hydrolysate from Acidic Pretreatment of Biomass in a Mesophilic Anaerobic Fermentation System for the Production of Methane: Comparison of the Standard CSTR with Up-Flow Packed Bed Reactors," Biotech. Bioeng. Symp. 15:39-58.

Rivard, C.J., Bordeaux, F.M., Henson, J.M., and Smith, P.H. 1987. "Effects of Addition of Soluble Oxidants on the Thermophilic Anaerobic Digestion of Biomass To Methane," Appl. Biochem. and Biotech. (in press).

Suidan, M.T., Siekerka, G.L., Kao, S.-W, and Pfeffer, J.T. 1983. "Anaerobic Filters for the Treatment of Coal Gasification Wastewater," Biotech. Bioeng. 25:1581-1596.

Tzavaras, J., and Shendrikar, A.D. 1984. "An ICP Analytical Scheme to Screen Environmental Samples for Metals," American Laboratory.

U.S. Environment Protection Agency, 1982. Inductively Coupled Plasma-Atomic Emission Spectrometric Method for Trace Element Analysis of Water and Wastes. Method 200:7.

Young, J.C., and McCarty, P.L. 1969. "The Anaerobic Filter for Waste Treatment," J. Water Pollut. Control Fed. 41:R160-R173.

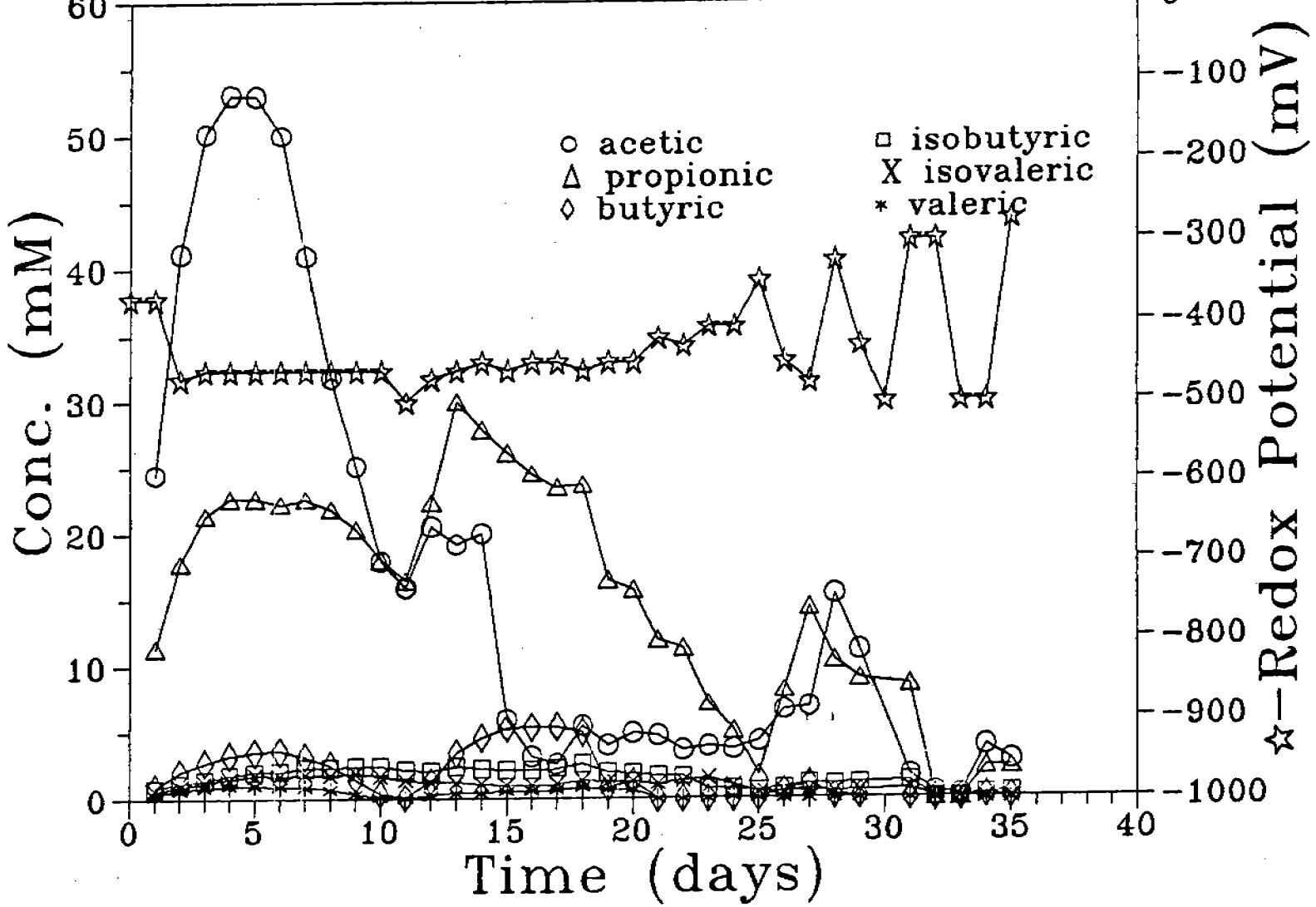


Figure 1. Volatile fatty acid levels and oxidation reduction potentials (Redox) over a during the first 35 days of digester operation on a municipal solid waste feedstock with added nutrients.

Wastewater Treatment
and
Landfill Gas Recovery

LANDFILL GAS GENERATION AND MIGRATION: REVIEW OF CURRENT RESEARCH

J. Bogner, C. Rose, M. Vogt, and D. Gartman
Argonne National Laboratory
Argonne, Illinois

ABSTRACT

Landfill gas research in the U.S. Department of Energy (DOE) Energy from Municipal Waste (EMW) Program is focusing on two major areas of investigation: 1) Landfill gas migration processes; and 2) Landfill gas generation. With regard to gas migration, a field investigation is examining bidirectional gas movement through landfill cover materials by processes of pressure and diffusional flow. The overall purpose of the study is to quantify gas loss from the landfill reservoir by natural venting and air influx due to pumping on recovery wells. Two field sites--a humid site with clay cover and a semiarid site with sand cover--have been instrumented to examine vertical gas movement through cover materials. Results from the humid site indicate that: (a) concentrations of methane, carbon dioxide, oxygen and nitrogen in soil gas vary seasonally with soil moisture; (b) based on average methane gradients in soil gas and a simple diffusion model, up to 10E5 g methane m^{-2} yr^{-1} are vented through the cover materials at the humid site (area of 17 ht); and (c) during prolonged wet weather, pressure gradients of more than 2 kPa may develop between the cover materials and top of refuse, indicating that pressure flow is periodically an important mechanism for gas transport.

The second project is addressing landfill gas generation. The major goal is to develop simple assay techniques to examine the gas production potential of landfilled refuse. Refuse samples extracted from various depths in a landfill are being leached by three different methods to separate microbial mass and substrate. The leachates are being subjected to Biochemical Methane Production (BMP) assays with periodic qualitative examination of microbial populations using fluorescence microscopy of live cultures and scanning electron microscopy (SEM). Triplicate assays of the leachates that produce insignificant quantities of biogas after 90 days incubation are being amended with sucrose, a nutrient broth, or a bacterial seed. Response of gas production to each of the three amendments was similar across all samples, regardless of the leaching method originally employed, with nutrient addition producing the most stable long-term biogas production with the highest methane content.

Work supported by the U.S. Department of Energy, Assistant Secretary for Conservation and Renewable Energy, under Contract W-31-109-ENG-38.

LANDFILL GAS GENERATION AND MIGRATION: REVIEW OF CURRENT RESEARCH

INTRODUCTION

The recovery of landfill gas is a commercial technology that provides locally significant supplemental energy. It is used commercially at approximately 60 sites in the United States as well as at sites in Great Britain, Europe, South America, and Asia. Landfill gas, the product of anaerobic decomposition of municipal solid waste (MSW), is a medium-Btu gas consisting of approximately half methane and half carbon dioxide. Currently, landfill gas is used as a medium-Btu fuel in existing industrial boilers and to generate electricity; at some sites, the gas is upgraded to a high-Btu gas by removing the carbon dioxide.

The economics of landfill gas recovery depend on user requirements, user proximity to the landfill, and sustainable gas recovery rates. The tipping fee (the cost of refuse disposal at a landfill) helps offset the capital costs of installing a gas recovery system and, depending on the size of the fee, may also help offset the capital costs of gas upgrading or electrical generation equipment. Currently, low natural gas prices are discouraging landfill gas upgrading for pipeline injection and favoring direct use.

Most landfill gas recovery systems are installed in large landfills (>9.0 x 10^8 kg of refuse) and consist of vertical pipes or horizontal trench collector systems. Economic recovery of landfill gas from smaller sites is dependent on a better understanding of gas generation and migration in situ. The existence of over 9,000 landfills in the U.S. suggests a substantial resource base that could be developed for energy production. The projects described herein are all directed toward maximizing the amount of gas ultimately recovered from U.S. landfills, particularly from the numerous smaller sites, by:

1) Better understanding methane loss and oxygen intrusion through cover materials, leading to recommendations to minimize these processes.

2) Increased understanding of gas generation in landfills, eventually leading to some degree of management of gas generation.

I. GAS MOVEMENT THROUGH LANDFILL COVER MATERIALS

BACKGROUND

In normal surface soils, molecular diffusion is generally more important than mass flow processes for exchange of soil gases with the atmosphere. Flux for a soil gas component is a function of a diffusion coefficient specific for a given gas diffusing in a porous medium, a concentration gradient for a given gas, and terms describing the porous medium (air-filled porosity, tortuosity). In a uniform soil profile, air temperature, wind speed, barometric pressure, and soil moisture fluctuations generally affect gas exchange

in only the top few centimeters of soil air (Baver, 1956; Pojasov, 1967; Currie, 1970; Kimball and Lemon, 1971; Perrier et al., 1974; Glinski and Stepniewski, 1985). Kimball (1983), reviewing the work of previous investigators, concluded that barometric pressure fluctuations can cause perturbations of up to 60% in soil gas diffusion rates only in "deep" soils (>100 m to water table or other barrier).

Concentrations of soil gas components outside of a landfill setting reflect a wide range of chemical, biochemical, and transport processes, including root-zone respiration, abiogenic redox reactions, and metabolism of microorganisms and megafauna. Oxygen and carbon dioxide, in particular, undergo regular diurnal and seasonal variations related to temperature and soil moisture influences on root-zone respiration (Currie, 1975). Soil gas typically consists of approximately 79% nitrogen (volume %) with the remaining 21% composed of carbon dioxide and oxygen, which vary reciprocally (Campbell, 1985).

Soil gas above decomposing refuse, on the other hand, exhibits elevated concentrations of methane and carbon dioxide and depressed concentrations of nitrogen and oxygen. With increasing depth in the soil column, pressures in excess of atmospheric may develop due to gas generation in the decomposing refuse. Hence, both pressure and diffusional flow processes are important. Gas movement through the cover materials would depend on the nature of the cover materials, the vegetative cover, and meteorological variables. Soil moisture changes, in particular, would drastically affect the permeability of the cover with regard to gas transport. Methanotrophs (methane-oxidizing bacteria) may also be important influences upon soil gas composition in landfill cover materials. Work by Mancinelli and McKay (1984) suggests that up to 10% of the methane produced in a landfill may be consumed by methanotrophs in near-surface aerobic zones.

METHODS

For gas recovery purposes, it would be desirable that all of the gas produced in a given landfill gas "reservoir" be available to the recovery system. Natural migration of gas out of the landfill, influx of air into the landfill, and consumption of gas by methanotrophs in the upper zones of the landfill restrict the amount of available gas at a given site. This study is concentrating on understanding rates and directions of gas movement through cover materials over time.

Gas flux through soil materials can be measured by three techniques: (1) chamber methods (either static or dynamic), in which flux of a particular component per unit time is measured by the increase in that component in a chamber placed on the soil surface; (2) micrometeorological (or aerodynamic) methods, which compute fluxes above or within the vegetative canopy from above-ground concentration gradients and estimates of the transfer coefficients, and (3) soil profile methods, which utilize below-ground concentration gradients and estimates of appropriate diffusion coefficients (deJong et al., 1979; Cionco, 1983; Lemon, 1983, Glinski and Stepniewski, 1983; Parrish et al., 1986).

The present study uses subsurface vertical technique, with supporting meteorological and soil moisture data, to measure both pressure and concentration gradients in the cover materials and top of refuse. This method was chosen to permit

study of bidirectional flow for four major soil gas components in the landfill cover; these experiments include CH_4 and CO_2 (the major components of landfill gas) and N_2 and O_2 (the major components of air).

In a landfill setting, both diffusion and pressure flow are important to gas migration through the cover. Air and landfill gas mix in gas-filled pore spaces in the cover materials. In the field, the concentrations of the four gases and soil gas pressures are being monitored at several depths and at several locations on two landfill sites--a humid site (DuPage County, Illinois) with clay cover and a semiarid site (Orange County, California) with sand cover. Meteorological variables, soil moisture, and soil temperature are also being monitored during the course of the study to quantify relationships between these variables and the vertical pressure and concentration gradients.

From the vertical pressure and concentration gradients, it is possible to calculate pressure flow for total gas (Darcy) and diffusional flux for individual gas components (Fick), using representative values for physical properties of soil. The resulting data are being analyzed to examine the rates and direction of gas flux through landfill cover materials in response to both natural meteorological changes and pumping rates at adjacent recovery wells.

The probe design is shown in Fig. 1 and consists of a thin stainless steel tubing inside an outer PVC casing; a T-connection at the top of the tubing permitted installation of both an electronic pressure transducer (via a quick-connect air compressor hose connection) and a gas tubing/septum port for withdrawal of gas samples with a hypodermic syringe. Probes are purged of dead air before samples were withdrawn for analysis. The pressure transducers from each nest and various weather station sensors are linked to an RCA COSMAC microcomputer equipped with a cassette tape I/O device for continuous monitoring of soil gas pressures, barometric pressure, and other meteorological variables (wind speed, wind direction, temperature, precipitation, and solar radiation). A field gas chromatograph (GC) permits frequent analysis for oxygen, nitrogen, methane, and carbon dioxide. A Micromonitor GC equipped with Chromosorb 102 and M.S. 13A columns is being used; the unit is calibrated with standard gas mixtures. All analyses were done in triplicate. Soil moisture is determined by tensiometers and down-hole neutron probe methods (Troxler equipment). Soil temperature is measured by arrays of thermister probes installed at various depths.

RESULTS AND DISCUSSION

Field work at the humid site was completed in August, 1987; field work began at the semiarid site in late October, 1987. The results presented below pertain only to the humid site, the Mallard North Landfill in northern DuPage County, Illinois, about 50 Km west of Chicago. This site covers approximately 17 hectares and consists of mounded refuse 10-20 m thick capped with a 1.2-2.5 m thick clay cover.

Figure 2 shows average methane concentrations in soil gas for probes located on top of the Mallard North site (1A-1D; 4A-4D) and probes located on the side slopes (2A-2D; 3A-3D). Both methane and carbon dioxide concentrations were much higher in soil

gas on the side slopes, where greater fracturing of the clay cover was observed. In general, concentration profiles for all gases steepened sharply at the refuse/cover boundary; Fig. 3 shows methane concentration profiles for probe nest 2 (PN2) and probe nest 3 (PN3). Using the average methane concentrations in Fig. 2 to define methane gradients and Fick's First Law to calculate flux, considering molecular diffusion only (Lehman, 1979), an average methane flux through the cover can be calculated:

$$F = - D_{12} \frac{dC}{dz}$$

where:

F = flux, g cm^{-2} sec^{-1},

D_{12} = bulk diffusion coefficient of CH_4 in soil air at 15°C and NP; friable clay soil with porosity = 0.6 (Lehman, 1979).

$\frac{dC}{dz}$ = concentration gradient, g cm^{-3} cm^{-1}.

Results suggest rates ranging from 10^{-8}g cm^{-2} sec^{-1} to 10^{-6} g cm^{-2} sec^{-1} for the Mallard North site, with the higher rates on the side slopes.

Examining changes in soil gas concentrations through time, methane and carbon dioxide generally have parallel plots as do oxygen and nitrogen (Fig. 4). Methane and carbon dioxide concentrations increase and oxygen and nitrogen decrease during times of heavy precipitation (also Fig. 4). This is due to the "sealing off" effect of increased soil moisture (diminished gas-filled space) and possibly to increased gas generation rates during wet periods.

Soil gas pressure gradients develop between the upper part of the refuse and the cover materials during times of heavy precipitation and cold weather (freezing ground conditions). As shown in Fig. 5 for cold weather and freezing ground during late fall, 1985, differential pressures may exceed 2 kPa, indicating that pressure flow mechanisms are seasonally important for gas transport. In general, soil gas pressures vary with barometric pressure, both diurnally and in response to passage of major pressure fronts.

CURRENT AND FUTURE WORK

A one-dimensional finite difference formulation for combined pressure and diffusional flow has been developed. It is being tested with representative field data from Mallard North to examine its sensitivity to various input variables.

Analysis of Mallard North data is continuing, concurrent with start-up of work at the Olinda Landfill, northeastern Orange County, California. The Olinda site has a 1 m silty sand cover in its Alpha Canyon area; this site was chosen to provide a sharp contrast to Mallard North in terms of climate (semiarid) and type of cover (sand). Four probe nests have been installed at Olinda; each nest includes five probes at depths ranging from 20 cm to 330 cm. Work at Olinda will continue until mid-summer, 1988, to monitor both dry and wet weather conditions. The probe nests are spaced around a proposed gas recovery well; it is planned that monitoring of soil gas pressure and concentration changes during pumping will be possible during the early summer, 1988. This was not possible at the Mallard North site. Also, direct monitoring of methane flux out of the cover (by area techniques) is planned to compare with rates calculated by gradient techniques. The results of this project will be used to recommend tighter cover designs and seasonal pumping schedule alterations that would be cost-effective to limit methane loss and oxygen influx. In addition, a better understanding of vertical gas transport processes, landfill mass balance relations, and improved predictive capability for gas recovery systems will be gained.

II. GAS GENERATION POTENTIAL AND QUALITATIVE MICROBIOLOGY OF LANDFILLED REFUSE

BACKGROUND

At the present time, sustainable gas recovery rates at a particular landfill site are derived from field pumping tests and assumptions regarding gas generation rates. However, actual production rates at many sites are lower than originally expected; treatment plants may thus be over-designed and operating at some fraction of full capacity. Previous work on landfill gas generation, including numerous laboratory studies (Halvadakis, Robertson, and Leckie, 1983; Jenkins and Pettus, 1985; Emberton, 1987; Barlaz, Milke, and Ham, 1987) and one large-scale field study (Pacey and Dietz, 1987) are inconclusive with regard to the value of adding nutrient, buffer, and bacterial seed to promote biogas production from refuse. In large part, inconsistencies result from the lack of standardized procedures to assay gas production from landfilled refuse. Optimization of moisture content appears to be important since water is the major transport medium in a landfill setting; Rees (1985), for example, demonstrated increased rates of gas production below the water table at the Aveley landfill (England). Indeed, landfill leachate (produced by water percolating through decomposing refuse) contains high concentrations of volatile fatty acids (VFA's), the intermediate decomposition products of acetogenic bacteria and the presumed substrate for methanogenic populations.

The studies cited above suggest that high rates of gas production (10^{-1} m^3/kg/yr or more) can be attained; however, basic understanding of the microbial ecology of landfills and standardized techniques to evaluate gas production potential of landfilled refuse are generally lacking. A major goal of this current study is to begin to develop simple, standardized field and laboratory techniques for evaluating gas production from landfilled refuse. In particular, we are testing the application of Biochemical Methane Potential (BMP) assay procedures, commonly used in evaluation of anaerobic biodegrad-

abilty of industrial wastewaters, as a possible tool to evaluate landfill gas production. This technique presumes an aqueous substrate, so we are using laboratory leachates of refuse samples collected from several depths at a local landfill. The use of laboratory leachates thus maximizes moisture to the refuse and permits the study of other variables.

In addition, we are testing several techniques to qualitatively examine the microbiology of the resulting assays, including fluorescence microscopy of live cultures and scanning electron microscope (SEM) techniques. Quantitative techniques to examine methanogenic populations in landfilled refuse are currently being developed elsewhere (Peck, 1987; D. Archer, 1987; personal communication). Since the microbial ecology of landfills is complex, including hydrolytic, acetogenic, and methanogenic organisms, a practical first step is to better understand gas production in situ via standardized sampling and assay procedures and, secondarily, qualitatively examine microorganisms in the landfill assays.

A better understanding of controls on landfill gas generation will permit some degree of management of gas production at specific sites to fit user requirements. This is particularly important at smaller landfill sites. The overall goals of managing gas production in landfills include rapid initiation of gas production, high methane content in biogas (50% or more), and stable rates of gas production over time to fit user requirements.

METHODS

Approximately 60 refuse samples were collected from depths ranging from 10-120 feet at the Greene Valley Landfill, DuPage County, Illinois. The samples were collected by hand from the center of refuse cores extracted by cylindrical bucket auger with overall dimensions of approximately 1 m (diameter) x 1.6 m (length). Measured internal temperatures ranged from 30-35°C. The samples were immediately placed in heavy-gage plastic bags and sealed after excess air was manually squeezed out of bags. All sample bags were kept at 4°C until analysis. Refuse age, based on recovered newspaper fragments, ranged from 6-12 years.

Visual classification of the refuse samples suggested three different types, as follows:

1. dry refuse: paper, leaves, grass, and some dry soil;
2. moist mixed refuse: paper, leaves, plastic, grass, metal, and soil;
3. grey slurry: wet clay and highly decomposed organic matter.

Three separate samples were randomly selected from each of the 3 refuse types (9 total samples). Random grabs of refuse were taken from three different areas in the sample bags to equal 50 g total for each sample. All sample handling and leaching procedures were performed in a Coy anaerobic chamber maintained with N_2:CO_2:H_2 (80:15:5). Total and volatile solids were determined for each sample.

Two simple leachate techniques were developed which varied in their severity to extract microbial mass and substrate from refuse. Each 50 g sample was split; each half was leached by one of the following procedures:

1. Water Wash Technique - Twenty-five g of refuse was loosely packed in a glass column and 300 mL of distilled water was poured through the column. The resulting leachate was then recirculated once through the column followed by a final rinse with 150 mL of distilled water.

2. Water Slurry Technique - Twenty-five g of refuse sample was ground with 300 mL of distilled water in a Waring blender for 20 seconds. The slurry obtained was then gravity-filtered through a Buechner funnel lined with a double layer of cheesecloth and rinsed with 150 mL of distilled water.

Both techniques resulted in approximately 450 mL of leachate. pH's of the resulting leachates were measured and all leachates were analyzed for alkalinity, volatile fatty acids (VFA's), and Chemical Oxygen Demand (COD) using standard methods.

The Biochemical Methane Potential (BMP) assay is a simple and inexpensive procedure developed for evaluation of biodegradability and possible toxicity of constituents in feed sources to anaerobic treatment processes (Owen, et al., 1979). In this study, standard BMP assays and modifications of the BMP procedure (to include the addition of specifically adapted anaerobic inocula, nutrient-rich broth, and/or a carbon substrate) were used to evaluate the refuse leachate for biodegradation potential. The general assay procedures were the same as those described elsewhere (Owen, et al., 1979), except that, initially, no nutrient media or anaerobic bacterial inocula were added in order to examine the gas production potential of the unamended refuse leachate. All transfers of the leachate to 125 mL serum bottles were performed in the anaerobic chamber. The bottles were sealed with 1-cm thick butyl rubber stoppers and capped with aluminum crimp seals. All test assays were done in triplicate and incubated at 33°C. Biogas generation was measured and expelled daily using a water-lubricated 10cc syringe equipped with a 19 gauge needle. The serum bottles were shaken vigorously before the measurements were taken. The methane production of the assay was quantified by injecting 0.5 mL of headspace gas from the serum bottles into a gas chromatograph (HP-5480A) equipped with a thermal conductivity detector.

After 90 days of incubation the assays that produced an insignificant volume of biogas were further treated by the addition of inocula, sucrose, or nutrient broth. A volume of 10 mL of liquid was removed from each assay bottle using a 10cc disposable syringe equipped with a 19 gauge needle. One of the triplicate test assays from each set of samples was injected with 10 mL of an anaerobic seed inocula. The anaerobic inocula consisted of landfill leachate from Blackwell Forest Preserve Landfill in DuPage County, Illinois which had been incubated anaerobically (BMP assays) at Argonne and demonstrated high rates of gas production. Laboratory work by Barlaz, Milke, and Ham (1987) indicated increased rates of gas production from refuse amended by old landfill leachate, presumably by addition of well-adapted methanogens. Refuse placement at the Blackwell site was completed in 1972; gas production is still occurring at the site as evidenced

by emissions from vertical vents placed in the fill. The second test assay from each set of samples was injected with 10 mL of sucrose solution to equal a final concentration of 1000 mg/L of assay volume. Sucrose was added as an easily degradable substrate in an attempt to elicit anaerobic decomposition of the refuse through co-metabolism. The third assay was treated with 10 mL of a concentrated nutrient broth described by Shelton and Tiedje, 1974. The defined media contains phosphate buffer, mineral salts, and trace metals for mixed anaerobic cultures and has been used routinely as nourishment for anaerobic inocula in assay testing at Argonne. After the assays were further incubated to 33°C, the bottles were vented to atmospheric pressure, and monitoring of biogas generation and methane composition continued.

RESULTS AND DISCUSSION

Sample descriptions and the leachate analyses are given in Table 1. Generally, the results on the leachates from both methods were similar. However, the samples treated by grinding and rinsing the refuse (water slurry) contained a substantially higher soluble COD value in the leachate than those same samples when treated by merely rinsing the refuse with water (water wash) Table 2 presents the results of the initial unamended BMP assays in terms of the average total biogas production and methane composition of the headspace and compares the two leaching techniques used for this study. The assays were initially incubated for 90 days or until biogas production had ceased. Since the organic substrate present in each sample was variable (determined by soluble COD concentrations), the values for the theoretical biogas production were based on 0.35 mL of methane/mg COD added and corrected for the solubility at the increased temperature. Leachates from the wet gray slurry samples were determined to have little or no soluble COD and produced no methane during the 90 days of incubation. However, leachates from some of both the dry and the moist refuse samples were observed to convert some, and in several cases, all of the soluble COD present in the sample. The water wash leachates produced slightly better results overall, in terms of bioconversion efficiency, than those treated by grinding and rinsing.

After 90 days of incubation those samples that produced little (less than 5% of expected theoretical) or no biogas were selected for the co-treatment methods previously discussed. Leachates from the gray slurry samples which had produced negligible biogas initially in the unamended assays, continued to produce negligible biogas after the addition of inocula, sucrose, and nutrient broth. It would appear that the gray slurry samples were highly decomposed and did not contain enough residual organic substrate in a readily available form to support active anaerobic microbial populations.

The results of the co-treatment methods used to enhance the biodegradation of the dry and moist refuse samples are presented in Table 4. The addition of anaerobic inocula to the assays resulted in rapid initiation of biogas generation. However, all of the observed biogas generation was complete within 14 days after injection; also, the gas was not of a high methane composition and overall bioconversion efficiencies were not increased as substantially as with the other co-treatment methods discussed below. The addition of 1000 mg/L sucrose solution resulted in rapid initiation of methane production in the assays, a stable production of gas over 55 days of incubation, and a high methane

content in the gas generated. The bioconversion efficiency of the assays was substantially increased in the presence of a readily available carbon substrate. The results indicated that populations of methanogenic bacteria were present on the refuse but were not in an active form, able to biodegrade the organic matter leached from the refuse. The addition of the nutrient broth produced the greatest increase in the bioconversion efficiency in the assays of dry and moist refuse. Figure 6A shows the percent bioconversion efficiency of each assay sample prepared by the water-wash technique treated with inocula, sucrose, and nutrient broth. Figure 6B shows the results of the same treatments on the samples prepared by the water-slurry technique. In terms of bioconversion efficiency over the 55-day incubation, the order of increasing efficiency of the co-treatments was consistent: e.g., nutrient broth addition greater than sucrose, sucrose addition greater than inocula; inocula addition greater than unamended refuse.

Results from this study indicate that methanogenic bacterial populations are present within landfilled refuse. Moreover, optimization of moisture as a transport medium plus the addition of suitable amendments can promote stable production of biogas with a high methane content. For assaying refuse at specific landfill sites, the BMP method shows initial promise. Even though the microbial ecology of landfills is complex, it appears possible to use simple standardized assay procedures to evaluate gas production potential at existing landfills to provide guidance for design of recovery systems which more closely match gas production. Particularly at landfills in humid climates (increased moisture), the results of this study suggest that the addition of suitable amendments to promote more rapid biodegradation and higher rates of gas production should be investigated more fully in additional laboratory and field testing. Other sites, for example, may have somewhat different requirements for increasing biodegradation. Attention should be focused on more precise elucidation of the microbial ecology of landfill organisms. Ultimately, it may be possible to design landfills with permeable vertical and horizontal corridors for better moisture circulation and provide suitable nutrient, buffer, and inocula to initiate rapid gas production commensurate with requirements of a specific user.

CURRENT AND FUTURE WORK

The initial set of BMP assays described above are being repeated with the following additions:

1. A third leaching technique - refuse:water mixture shaken with glass beads and gravity-filtered; BMP assay of filtrate.

2. BMP assay of refuse in distilled water (no leaching).

3. Co-treatments expanded to include component fractions of nutrient broth (Shelton and Tiedje, 1974) previously employed.

4. Periodic fluorescence microscopy of live cultures and periodic scanning electron microscopy of fixed-films placed in replicate assay bottles.

Future work will include more extensive BMP assay testing of samples from several landfills. The most promising techniques will undergo further testing in bench-scale gas generation experiments. Ultimately, a field experiment at a gas recovery site is planned.

ACKNOWLEDGMENTS

Project funding has been provided by the U.S. Department of Energy, Assistant Secretary of Conservation and Renewable Energy, Division of Biofuels and Municipal Waste Technology, under Contract W-31-109-ENG-38,

The cooperation and assistance of the Forest Preserve District (FPD) of DuPage County, Illinois, is gratefully acknowledged. We would also like to thank numerous personnel of the Orange County, California, General Service Administration, Solid Waste Division. The assistance of the following Argonne personnel is gratefully acknowledged: Michael Torpy, David Gartman, Ken Brubaker, Conrad Tome, John Taylor, and Stanley Zellmer. Finally, we would like to thank Charles Moore, Geotechnics, Inc., Columbus, Ohio, for advice regarding pressure transducers and microprocessor control units.

REFERENCES CITED

Archer, D., 1987, Institute of Food Research, Norwich, U.K., personal communications.

Barlaz, M.A., M.W. Milke, and R.K. Ham, 1986, *Parameters Affecting Refuse Methanogenesis and the Solids Composition of Anaerobically Degraded Refuse*, Proc. Ninth Annual Madison Waste Conf., Sept. 1986, Dept. of Engineering Professional Development, University of Wisconsin, Madison, Wis.

Baver, L.D., 1956, Soil Physics, (3rd ed.), J. Wiley & Sons, New York.

Cionco, R.M., 1983, *Canopy Gas Exchange: Canopy Flow Modeling and its Applications*, in Limitations to Efficient Water Use in Crop Production, American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, Wisconsin.

Campbell, G.S., 1985, *Soil Physics with Basic: Transport Models for Soil-Plant Systems*, Elsevier, New York.

Currie, J.A., 1970, *Movement of Gases in Soil Respiration*, in Sorption and Transport Processes in Soil, Society of Chemical Industry Monograph No. 37, London.

Currie, J.A., 1985, Tech. Bull. Min. Agric., Fish, Food, 29:461 (1975) quoted in Glinski and Stepniewski.

deJong, E., Redmann, R.E., and Ripley, E.A., 1979, *A Comparison of Methods to Measure Soil Respiration*, Soil Science, 127:300-306.

Glinski, J., and Stepniewski, W., 1985, *Soil Aeration and Its Role for Plants*, CRC Press, Inc., Boca Raton, Florida.

Jenkins, R.L., and J.A. Pettus, 1985, *The Use of In-Vitro Anaerobic Landfill Samples for Estimating Landfill Gas Generation Rates*, Proc. First Symposium on Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals, A. Antonopoulos, ed., Aug. 1984, Minneapolis, Minn., Argonne National Laboratory Report ANL/CNSV-TM-167.

Kimball, B.A., and Lemon, E.R., 1971, *Air Turbulence Effects Upon Soil Gas Exchange*, Soil Science Soc. of America Proc., 35:16.

Kimball, B.A., 1983, *Canopy Gas Exchange: Gas Exchange with Soil*, in Limitations to Efficient Water Use in Crop Production, American Society of Agronomy, Crop Science Society of America, and Soil Society of America, Madison, Wisconsin.

Lemon, E., 1983, *Canopy Gas Exchange: An Overview*, in Limitations to Efficient Water Use in Crop Production, American Society of Agronomy, Crop Science Society of America, and Soil Science of America, Madison, Wisconsin.

Lerman, A., 1979, *Geochemical Processes-Water and Sediment Environments*, Wiley-Interscience, New York.

Mancinelli, R.L., and C.P. McKay, 1985, *Methane-Oxidizing Bacteria in Sanitary Landfills*, Proc. First Symposium on Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals, A. Antonopoulos, ed., Aug. 1984, Minneapolis, Minn., Argonne National Laboratory Report ANL/CNSV-TM-167.

Owen, W., Stuckey, D., Healy, J., Young, L., and P. McCarty, 1979, *Bioassay for Monitoring Biochemical Methane Potential and Anaerobic Toxicity*, Water Res. Vol. 13, 485-492.

Pacey, J.G., and A.M. Dietz, 1986, *Gas Production Enhancement Techniques*, preprints Energy from Landfill Gas Conference held October, 1986, Solihull, West Midlands, U.K.

Parrish, D.D., et al., 1987, *Measurement of Nitrogen Oxide Fluxes from Soils: Intercomparison ofEnclosure and Gradient Measurement Techniques*, J. Geophysical Research, 92:1265-2171.

Peck, M.W., 1986, *Indirect Methods for the Enumeration and Identification of Methanogenic Bacteria in Landfill Samples*, in Energy from Landfill Gas, J.R. Emberton and R.F. Emberton, eds., Proc. of Conference held October, 1986, Solihull, West Midlands, U.K.

Perrier, A., Archer, P., and Planco de Pablos, 1974, *Study of Real and Maximum Evapotranspiration of Various Crops*, Ann. Agronomy, 25:697.

Pojasov, N.P., 1967, *Soil Aeration*, in The Basis of Agrophysics (Polish) Joffe, A., and Revat, Z., eds., Warsaw (1967) quoted in Glinski and Stepniewski (1985).

Rees, J., 1985, *Chemical and Microbiological Analysis of Aveley Landfill, England*, Proc. First Symposium on Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemical, A. Antonopoulos, ed., Aug. 1984, Minneapolis, Minn., Argonne National Laboratory Report ANL/CNSV-TM-167.

Shelton, D. and J. Tiedje, 1974, *General Method for Determining Anaerobic Biodegradation Potential*, Appl. Environ. Microbiol. 47 (4), 850-857.

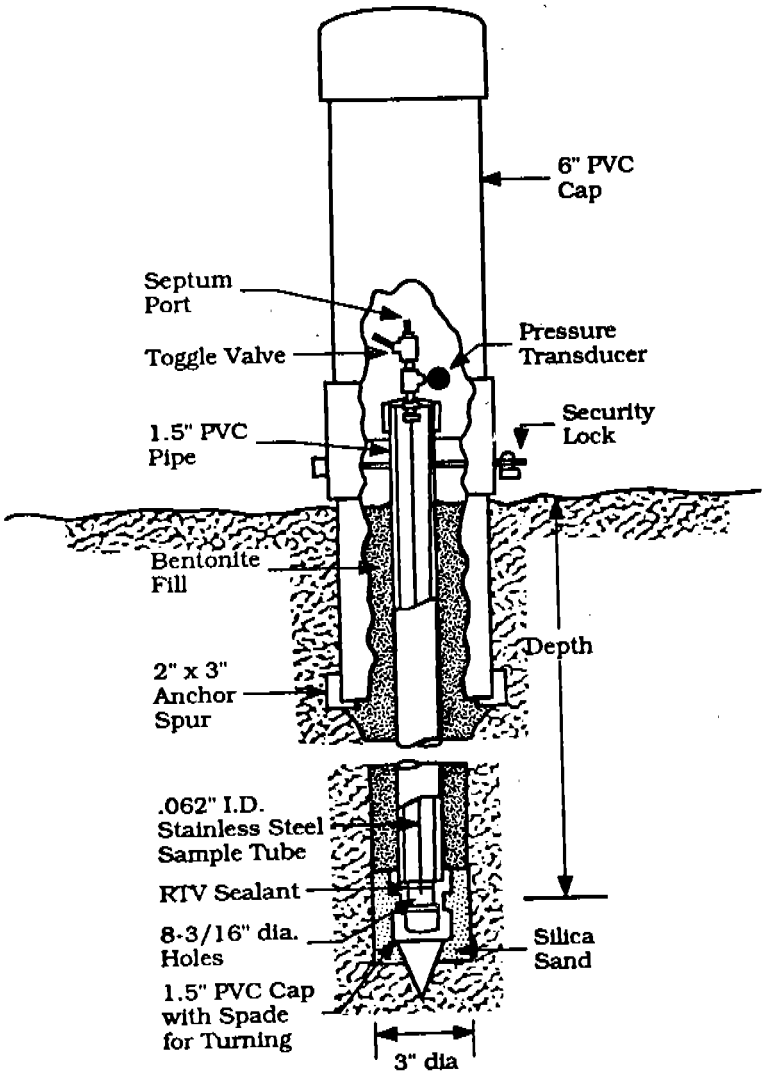


Figure 1. Schematic of soil gas monitoring probe

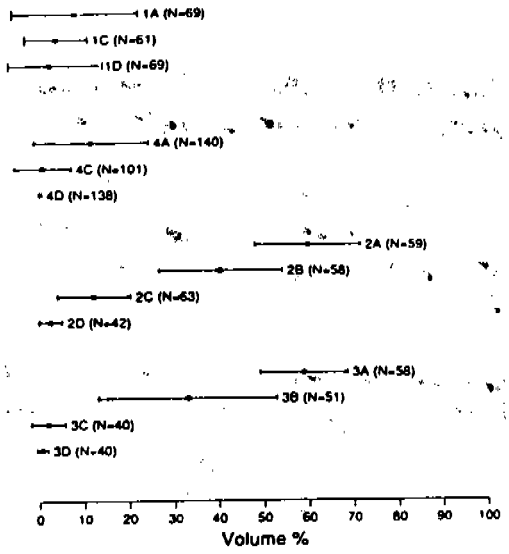


Figure 2. CH_4 concentrations in soil gas at Mallard North Landfill. Mean values ±1 standard deviation for period 4/86 - 7/87. Probes 1A-1D and 4A-4D on top of landfill mound; probes 2A-2D and 3A-3D on side slopes. Probes 1A, 2A, 2B, 3A, 3B, 4A in top of refuse (1.2-2.7 m deep). Probes 1C, 2C, 3C, 4C, in cover (1.0-1.3 m deep). Probes 1D, 2D, 3D, 4D in cover (0.6-0.7 m deep).

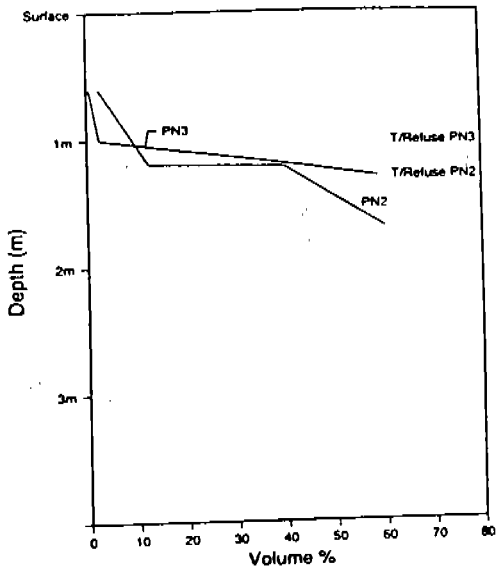


Figure 3. CH_4 concentration profile for probe nest 2 (PN2) and probe nest 3 (PN3) located on Mallard North side slopes. Mean values for period 4/86 - 7/87.

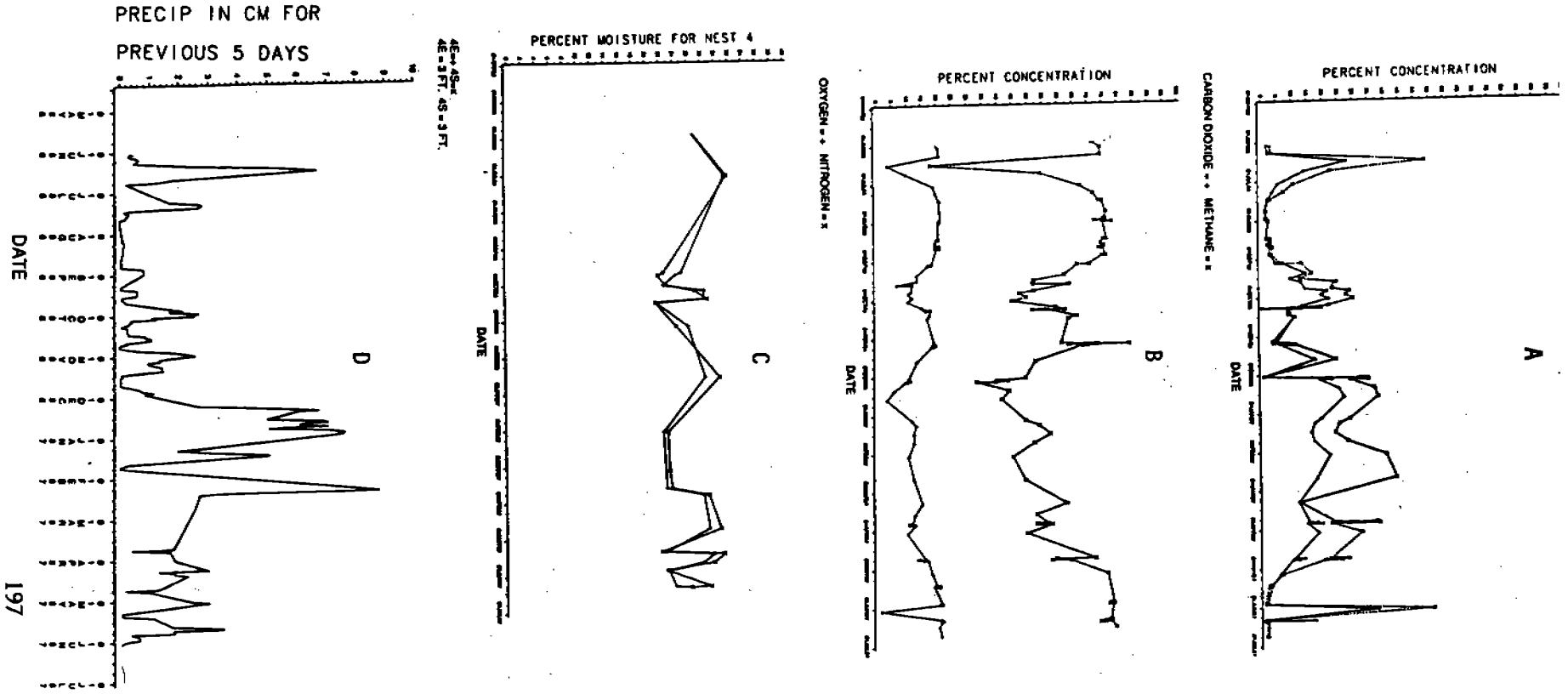


Figure 4. Soil gas concentrations, soil moisture and precipitation relationships for period June, 1986 - July, 1987.

A. Carbon dioxide and methane (V%) for probe 4A (1.7 m deep).
B. Oxygen and nitrogen (V%) for probe 4A (1.7 m deep). Y-axis 0-100%.
C. Soil moisture (%) at 1 m. Y-axis 0-40%; most values 22-30%.
D. Cumulative rainfall expressed as sum of precipitation over previous 5 days (cm).

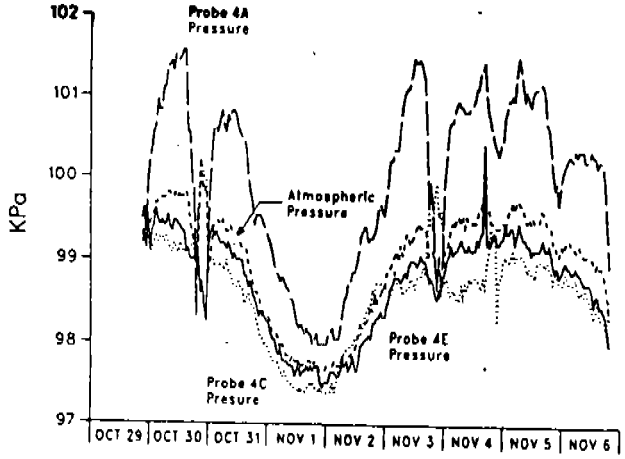


Figure 5. Atmospheric pressure and soil gas pressures in probe nest 4 vs. date (1985). Probe 4A is 1.7 m deep; 4C is 1.3 m deep, 4E is 0.7 deep.

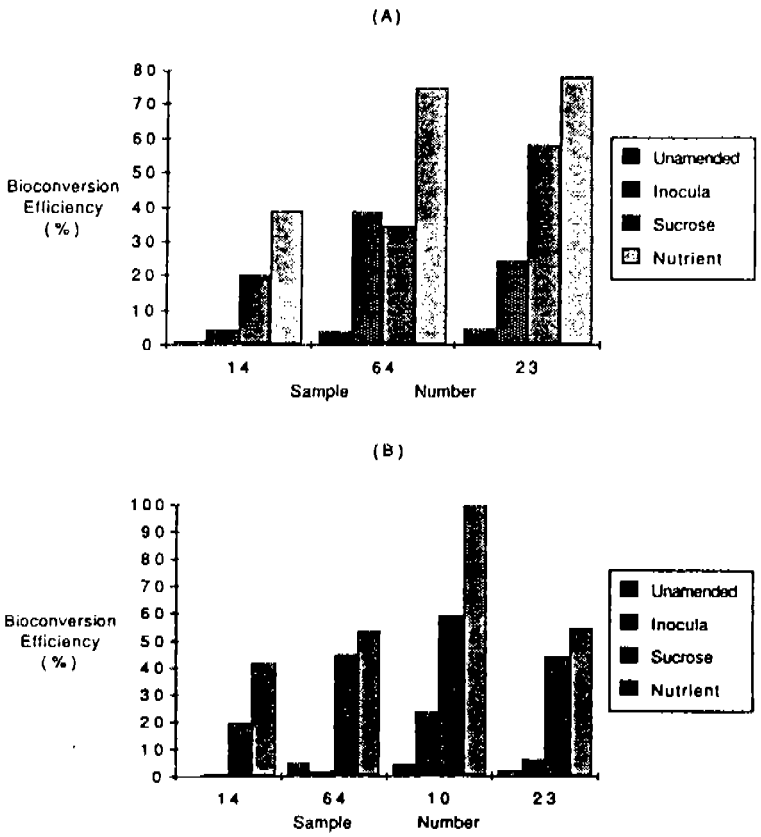


Figure 6. Results of co-treatment expressed as % bioconversion efficiency. 55 days incubation after addition of co-treatment.
A. Water-wash method
B. Water-slurry method

Table 1. Description of samples and chemical characteristics of leachates

Sample Description					Chemical Characteristics							
					Water Wash Method				Water Slurry Method			
Sample Numbers	Description	Depths	Total Solids (%)	Volatile Solids (%)	pH	Alkalinity (mg/L)	VFA (mg/L)	COD* (mg/L)	pH	Alkalinity (mg/L)	VFA (mg/L)	COD* (mg/L)
14	paper,leaves,soil (dry)	50 ft	85.2	83.6	5.7	220	190	763	5.7	480	170	2032
55	paper,leaves,soil,plastic (dry)	40 ft	99.7	77.3	6.8	270	190	549	5.8	470	400	1789
64	paper,leaves,soil,plastic (dry)	10 ft	66.9	90.2	5.7	320	240	1037	5.5	590	350	2800
10	paper,grass,wood,pulp (moist)	10 ft	72.2	42.1	5.7	420	340	817	5.7	660	350	1037
23	paper,grass,wood (moist)	30 ft	53.1	80.9	6.6	740	200	2282	6.6	1210	340	4231
53	moist paper,plastic,soil (moist)	50 ft	53.5	79.4	6.4	330	270	1093	6.0	1000	760	3070
48	gray clay slurry (wet)	20 ft	64.1	16.1	7.9	200	0	0	7.9	3000	0	0
49	gray clay slurry (wet)	30 ft	48.6	37.6	8.0	560	0	0	8.1	1650	0	134
74	gray clay slurry, paper (wet)	40 ft	83.3	4.5	7.8	230	0	0	8.0	5990	50	0

* Soluble COD values

Table 2. Results of initial BMP assays

Leaching Technique	Refuse Type	Sample Number	Sample COD (mg/L)	Theoretical * Biogas Production (mL)	Actual Biogas** Production (mL)	Methane Composition (%)	Bioconversion Efficiency (%)
Water Wash	Dry Refuse	14	763	80.6	0.8	0	1.3
		55	549	43.6	54.8	24	100.0
		64	1037	82.4	3.3	0	4.0
	Moist Mixed Refuse	10	817	64.9	66.4	41.5	100.0
		23	2282	90.7	4.3	0	4.7
		53	1093	86.9	17.0	9.8	19.6
	Wet Gray Slurry	48	0	0	0	0	- -
		49	0	0	0.7	0	- -
		74	0	0	0.7	0	- -
Water Slurry	Dry Refuse	14	2032	161.5	0.4	0	0.3
		55	1789	142.2	117.3	44.9	82.5
		64	2800	222.6	10.7	1.2	4.8
	Moist Mixed Refuse	10	1037	82.4	3.7	0.1	4.5
		23	4231	336.3	6.4	0.3	1.9
		53	3070	244.0	106.6	56.5	43.6
	Wet Gray Slurry	48	0	0	0.3	0	- -
		49	134	10.6	2.2	0	- -
		74	0	0	0	0	- -

* Theoretical Biogas Production estimated from 0.35 mL methane/mg COD added and corrected for biogas solubility

Table 3. Results of BMP assays after co-treatment

Leaching Technique	Refuse Type	Refuse Treatment	Sample Number	Theoretical# Biogas Production (mL)	Actual Biogas Production (mL)	Methane Composition (%)	Bioconversion Efficiency* (%)
Water Wash	Dry Refuse	unaltered leachate	14	60.6	0.8	0.0	1.3
		inocula *		60.6	2.6	0.7	4.3
		sucrose **		140.1	24.2	35.3	20.5
		nutrient broth ***		60.6	23.6	31.5	39.0
		unaltered leachate	64	82.4	3.3	0.0	4.0
		inocula *		82.4	32.2	0.4	39.1
		sucrose **		161.9	56.1	51.7	34.7
		nutrient broth ***		82.4	62.5	49.8	75.8
	Moist Refuse	unaltered leachate	23	90.7	4.3	0.0	4.7
		inocula *		90.7	22.3	0.4	24.6
		sucrose **		170.2	99.7	65.5	58.6
		nutrient broth ***		90.7	71.5	66.5	78.9
Water Slurry	Dry Refuse	unaltered leachate	14	161.5	0.4	0.0	0.2
		inocula *		161.5	1.4	0.0	0.9
		sucrose **		241.0	47.2	18.3	19.5
		nutrient broth ***		161.5	66.8	49.4	41.6
		unaltered leachate	64	222.6	10.7	1.2	4.8
		inocula *		222.6	5.0	2.0	2.2
		sucrose **		302.1	135.1	68.2	44.7
		nutrient broth ***		222.6	118.8	70.4	53.4
	Moist Refuse	unaltered leachate	10	82.4	3.7	0.1	4.5
		inocula *		82.4	18.6	8.9	23.6
		sucrose **		161.9	96.2	62.2	59.4
		nutrient broth ***		82.4	86.8	66.0	100.0
		unaltered leachate	23	336.3	6.4	0.3	1.9
		inocula *		336.3	21.0	0.3	6.2
		sucrose **		415.8	183.8	71.1	44.2
		nutrient broth ***		336.3	184.1	74.1	54.8

Theoretical Biogas Production estimated from 0.35 mL methane/mg COD added to BMP bottle, corrected for biogas solubility

* 10% (v/v) anaerobic seed (old landfill leachate) was added directly to the test assay

** sucrose was added in the concentration 1000 mg/L of test assay (additional COD corrected for in the theoretical biogas calculation)

*** solution of nutrients as perscribed by Shelton (1974)

EFFLUENT DEWATERING RESEARCH FOR MSW AND WASTEWATER TREATMENT SLUDGES

C. Victor Pearson
Manager Wastewater Research
Energy from Municipal Waste Program
Argonne National Laboratory
Argonne, Illinois 60439

ABSTRACT

Since 1983 the program for investigation of methods to achieve near energy independence in wastewater treatment plants has focused on two areas that have the greatest possibility for energy reduction and subsequent energy production in the processing of municipal wastewater. Development of a low energy process to produce combustor-fuel-quality sludge has been the primary focus of the program.

Two processes have been investigated namely, the BMI electro-acoustic process and the low-grade energy, contact-drier process being developed at Clemson University. Both processes have achieved solids concentrations of from 37 to 45% using no more energy than is required by conventional equipment to achieve 15 to 22% solids. The end-product is autogenous and appears to be capable of being burned for energy production as a single fuel and is able to achieve the desired 1500 to 1700°F flame temperature required for odor control.

Work supported by the U.S. Department of Energy, Assistant Secretary for Conservation and Renewable Energy, under Contract W-31-109-ENG-38.

EFFLUENT DEWATERING RESEARCH FOR MSW AND WASTEWATER TREATMENT SLUDGES

INTRODUCTION

The objective of this research is to develop alternative methods for the dewatering and drying of sludges to produce a fuel-quality product in an efficient, economic, and as practical a manner possible, using low grade energy.

In 1983 a request for proposal was announced in the Commerce Business Daily and 14 proposals for 12 different processes were received. After careful evaluation two processes were considered to offer the the best possibility for a universal solution to the problem of achieving significant net energy production in the sludge dewatering, drying and burning sludge disposal process.

Earlier DOE studies had shown that wastewater treatment plants typically consume over 40% of the energy used by a municipal government in providing services to its constituents. The study also showed that the combined processes for the dewatering, incineration and /or disposal of the residuals was the largest or close to the largest portion of the total energy consumption by the the plant.

The two processes chosen for this research program were selected because they appeared to offer the highest net yield of energy to energy input ratio and were processes that could be used any were in the country for plants processing less than 10 million gallons of wastewater per day. Solar drying was proposed but it was considered to have limited application. Also considered was the fact that the greatest need was for a process that could be used in the over 22,000 plants that process less than 10 million gallons per day of wastewater.

This research program has been conducted over the past four years although it has only been funded for three years. Consequently the progress has been sporadic since it has been necessary to put portions of the program on hold for extended periods until additional funding has become available.

DESCRIPTION

1. Electro-Acoustic Dewatering Process (EAD)

This process is one that had been under development by Battelle Memorial Institute, Columbus, Ohio. It had demonstrated significant improvement in dewatering of industrial sludge but had never been evaluated for wastewater treatment sludges especially anaerobic digester sludges. The ANL funded program to evaluate the process was conducted in two phases, i.e., Process Applicability Evaluation; and Evaluation of Effect of Key Sludge Properties on Dewaterability.

Phase 1 consisted of three tasks, namely, (a) Sludge Characterization; (b) Batch dewatering tests; and (c) Energy and Economic Analysis of a Reference Process. The batch dewatering test consisted of static tests to determine the effect of an electric field on sewage sludge and the effect of an acoustic field, both as single forces and in combination.

The effects of variability in the sludge properties with time, and the effect of dewatering time, applied voltage, pH, cake thickness, and the effect of vacuum (driving force used to replicate the normal mechanical forces used in dewatering in the modified belt filter-press) were investigated in both the electric field-effects studies and the acoustic field-effects studies. Table 1 lists the data obtained during each experiment.

Table 1. List of Properties Recorded in Static Tests

a. Initial Solids Concentration-wt.%.
b. Initial pH.
c. Initial weight for the test specimen unit volume.
d. Initial current flow in amps. with 50 volts applied across the sludge surfaces.
e. Ultrasonic frequency.
f. During the course of the experiment the following were measured at frequent intervals;
    i. Current in amps
    ii. Temperature of the sludge
    iii. Vacuum (always adjusted to 15-in. Hg)
    iv. Volume of filtrate
    v. Voltage
    vi. Ultrasonic power in watts
g. Post test measurements were made of the following;
    i. Final solids concentration
    ii. Dewatering time

During this phase of the program more than one hundred tests were run, 95% of which were conducted with anaerobic digestor sludges. Typical samples were 60 grams in weight although 90 and 120 gram samples were also used. Test parameters included the following:

Voltage; 10, 25 and 50 volts
pH; 5,7,and 8.5
Watts (ultrasonic) 10,15 and 30 watts
Initial Solids concentration; 1.5, 15, and 22%

Figure 1 shows a schematic of the test apparatus used.

Phase 2 of the program attempted to determine the cause of the variability in the Phase 1 test results. In phase 1 the experimentors had observed significant differences in the zeta potential between various sludges and had observed that this one factor had the

largest single correlation to the variation in dewaterability. Phase 2 was conducted in several subtasks namely; leaching studies to determine if the problem was with the filtrate or the solids; evaluation of the effect of aging on the sludge properties; and evaluation of the effect of the treatment process on zeta potential as the sludge is dewatered. The effect of pH and ionic strength of the sludge on dewatering performance were also studied.

The results of the program are discussed in the next section, Results.

2. Low-Temperature, Low-Pressure, Contact Dryer System

The second process investigated under this program was a concept proposed by Clemson's Environmental Engineering Department. This process used a low temperature, low pressure, contact dryer coupled to a heat pump to supply recycled and upgraded steam to the dryer. The net result was a high thermal efficiency system that requires only power to operate the rotating equipment. Figure 2 shows a schematic of the proposed process.

The test program was conducted in two phases namely; a static test phase and a dynamic, pilot scale test phase.

Phase 1 developed the data base on key properties involved in the dewatering and drying process. Samples were collected from three wastewater treatment plant and included anaerobic digestor sludge, activated waste sludge and raw primary sludge. Later samples included sludge from an industrial wastewater treatment plant. The sludges were characterized for their moisture content, volatiles content, capillary suction time, specific resistance (resistance to the flow of water through the matrix of solids), particle size distribution, elemental chemical constituents that were considered to have a detrimental effect on either the dewatering process or corrosion of the mechanical components, and the calorific value of the sludges.

Test were conducted in a vacuum desiccator so that three pressure conditions could be evaluated, namely; .1, .5, and 1.0 atmospheres. Drying rates were conducted over a range of temperatures, i.e., 25, 50, 75, and 100°C. The condensate was collected and analyzed. The results of this work was used to determine the test conditions for the phase 2 continuous flow tests and to verify that the filtrate that would ultimately be used as the working fluid in the heat pump, was sufficiently innocuous to allow commercial materials to be used.

Phase 2 consisted of the design, construction, and operation of a 2.3 meter long, .273 meter diameter contact dryer to allow testing of the significant engineering parameters. Clemson paid for the design and construction of the dryer and the associated pumps and drive systems in order that this equipment could be used in other research of interest to the university after the ANL program was completed.

The dryer has a steam jacket covering the cylinder that forms the drying chamber and all energy supplied to the drying process is supplied to this chamber. Figure 3 shows a schematic of the test apparatus.

Three different designs of auger were used in the tests to propel and agitate the sludge as it passed through the dryer. Three different residence times were used in the continuous flow tests. Since the actual residence time in the dryer is a function of the agitation and kneading of the sludge by the auger as the sludge is moved through the drying chamber the details of the hydraulic pattern provided by each design of auger had to be studied and a set of test conditions were developed for the combined heat transfer and hydraulics of the system for each type of auger.

Once the optimum conditions for the dryer system were developed a series of evaluations were conducted to determine the combustion properties of the end-product. The results of this program is also discussed in the following section titled Results.

RESULTS

Battelle Memorial Institute - Columbus Electroacoustic Dewatering Process

Tests showed that a combination of proper electric field and acoustic field allowed anaerobic digestor sludges that normally can only be dewatered to 15 to 17% solids in conventional equipment to be dewatered to 37 to 40% solids in the EAD process with no more energy than is required in the conventional equipment to achieve 15% solids. Optimum performance was achieved with an electric field of 50 volts and an acoustic field of 15 watts at 20 kHz. Figure 4 shows the optimum dewatering achieved and the optimum combination of fields.

Evaluation of key factors determining the performance of the process indicated that the zeta potential was the one characteristic that had the largest effect on determining the dewaterability of a sludge. In the case of anaerobic digestor sludges, the zeta potential was observed to decrease with time, and the correlation of ultimate dewaterability to the zeta potential of the sludge at the time of processing coincided closely with the age of the sludge. Figure 4 shows the comparison of performance of sludges that are 1 hr.old and 24 hrs. old.

Further evaluations of changes in properties of the sludge as it is dewatered indicated that the pH increases as the sludge is dewatered; which in turn, increases the zeta potential but the increase in zeta potential during dewatering is not great enough to override the effect of the increase in "dewatering resistance" as the solids concentration increases. The net result is the flattening of the dewatering curve with solids concentration as shown in Figure 4.

Clemson University - Low-Pressure, Low-Temperature Contact Dryer

The significant results from the Clemson research came in the second phase of the program. The sludges used in the dynamic pilot-scale drying tests used sludges that had already been dewatered by commercial equipment. Further dewatering-drying in the contact dryer at the two test temperatures (i.e., 65°F and 95°F temperature difference between mean sludge temperature and the walls of the dryer) produced results typical of that shown in Figure 5.

Correlation of the performance of the different sludge to their unique properties showed that the two characteristics that were most significant were the capillary suction time (CST) and volatile solids (VS). As can be seen from the figure, the sludge with the lowest CST and the highest VS achieved the maximum dryness for the same set of drying conditions. No attempt was made to quantify the affects of these properties separately. In general, the results of the Clemson study are what might be expected and are consistent with field experience with commercial equipment. The real significance of the results is the improved dryness of the end product that was achieved with heat transfer surface temperatures of only 275°F and 307°F, respectively.

As can be seen from the figure, the best performance was accomplished with an industrial sludge and the poorest performance was with the anaerobically digested sewage sludge.

DISCUSSION

The results from the two programs are considered to justify the original contention that there are alternative processes to the present methods used to densify and dispose of sewage sludge. These alternatives, low energy processes, can produce a fuel quality product capable of providing a significant net energy input to the waste water treatment process. Energy and mass balances and an economic analysis of the two processes indicated that if aerobic sludges are dryed to 33% solids or if anaerobic sludges are dryed to 37% solids these materials, with their volatilization temperatures of approximately 660°F, are autogenous and capable of producing the desired 1600°F gas temperatures required to assure destruction of odor producing gases. The 1600°F product of combustion temperature is quite capable of producing steam of sufficient quality and quantity to significantly reduce the demand for external energy to the plant while still providing the energy needed to drive the sludge dewatering/drying equipment or to provide an energy offset by supplying steam elsewhere in the plant as an offset for electricity required to operate the drive motors or other electric powered devices. Furthermore, the processes investigated are appropriate to the wastewater treatment plant processing from 100,000 to 10 million gallons per day of wastewater.

ACKNOWLEDGMENTS

The work presented in this paper was funded by Argonne National Laboratory as a part of the Department of Energy's larger program in Energy from Municipal Waste.

Work performed by Battelle Memorial Institute, Columbus, was conducted by H.S. Muralidhara, D. Ensminger, N. Senapati, K. Bhatti, and B.C. Kim under the direction of S.P. Chauhan.

Work performed by Clemson University was conducted by graduate students under the direction of T.M. Keinath, T.J. Overcamp, and J.C. Hester. T.M. Keinath is the department head and program manager.

REFERENCES CITED

American Society for Testing and Materials, 1984a, *Standard Test Method for Gross Calorific Value of Solid Fuel by the Adiabatic Bomb Calorimeter*, D2015-77, Philadelphia, Pennsylvania, 05.05:413-417.

American Society for Testing and Materials, 1984b, *Standard Test Method for Total Sulfur in the Analysis Sample of Coal and Coke*, D3177-82, Philadelphia, Pennsylvania, 05.05:413-417.

American Society for Testing and Materials, 1984c, *Standard Test Method for Chlorine in Coal*, D2361-83, Philadelphia, Pennsylvania, 05.05:346-349.

Bollinger, J., May 14, 1984, *New Filtration Concept Uses Electro Deposition*, J. of Chemical Engineering, pp. 38-39.

Cortis, C.B., 1931, *Electrical Dewatering of Clay Suspensions*, Am. Ceramic Soc. Journal, 14, pp 220-263.

Ellis, D., and Sunderland, J.G., 1977, *Dewatering Sewage Sludge by Electro-osmosis Part II - Scale-up Data*, NTIS Report PB-276-412.

Greyson, J., and Rogers, H.H., 1971, *Dewatering Sewage Sludge by Electro-osmosis*, 1971, in Advances in Water Pollution Research, edited by S.H. Jenkins, Vol. I, Pergammon Press, New York, New York.

Heath, L., and Demirel, T., November, 1984, *Pressurized Elector-osmotic Dewatering*, paper presented at Engineering Foundation Conference on Flocculation, Sedimentation and Consolidation, Sea Island, Georgia.

Jessup, R.S., 1960, *Precise Measurement of Heat of Combustion with a Bomb Calorimeter*, National Bureau of Standards Monograph No. 7.

Karr, P.R., III, August, 1976, *Factors Influencing the Dewatering Characteristics of Sludge*, Ph.D. Dissertation, Clemson University.

Kowalska, E., Chmura, K., and Bien, J., 1978, Ultrasonics, 16, pp 183-185.

Parr Instrument Co., 1981a, *Instructions for the 1241 Adiabatic Oxygen Bomb Calorimeter*, Moline, Illinois.

Sunderland, J.G., 1977, *Dewatering of Sewage Sludge by Electro-osmosis, Part I - Basis Studies*, NTIS Report PB-276-412.

Sunderland, J.G., November 23, 1982, *Electrokinetic Thickening*, IEEE Power Division Conference on Energy Saving Techniques for Product Drying and Water Removal of Electricity.

Svarovsky, L., 1977, *Introduction to Solid-Liquid Separation*, Butterworths, Inc.

Uhl, V.W., and Rott, W.L., June, 1962, *Indirect Drying in Agitated Units*, Chemical Engineering Progress, 58(6): 37-44.

Wakeman, R.J., July/August, 1982, *Effect of Solids Concentration and pH on Electro-filtration and Separation*, Filtration and Separation, pp 316-319.

Yamahata, Y., Izawa, H., and Hasama, K., 1984, *Experimental Study of the Application of Paddle Dryers for Sludge Cake Drying*, Proceedings of the 1983 World Drying Conference, Osaka, Japan, p. 719.

Yukawa, H., Kobiyashi, K., and Hakoda, M., 1980, *Study of the Performance of Electro-kinetic Filtration Using Rotary Drum Vacuum Filter*, J. Chem. Engr., Japan, 13(5), pp 390-396.

Yukawa, H., Yoshida, H., Kobiyashi, K., and Hakoda, M., 1976, *Fundamental Study on Electro-osmotic Dewatering of Sludge at Constant Electric Current*, J. Chem. Engr., Japan, 9(51), pp 402-407.

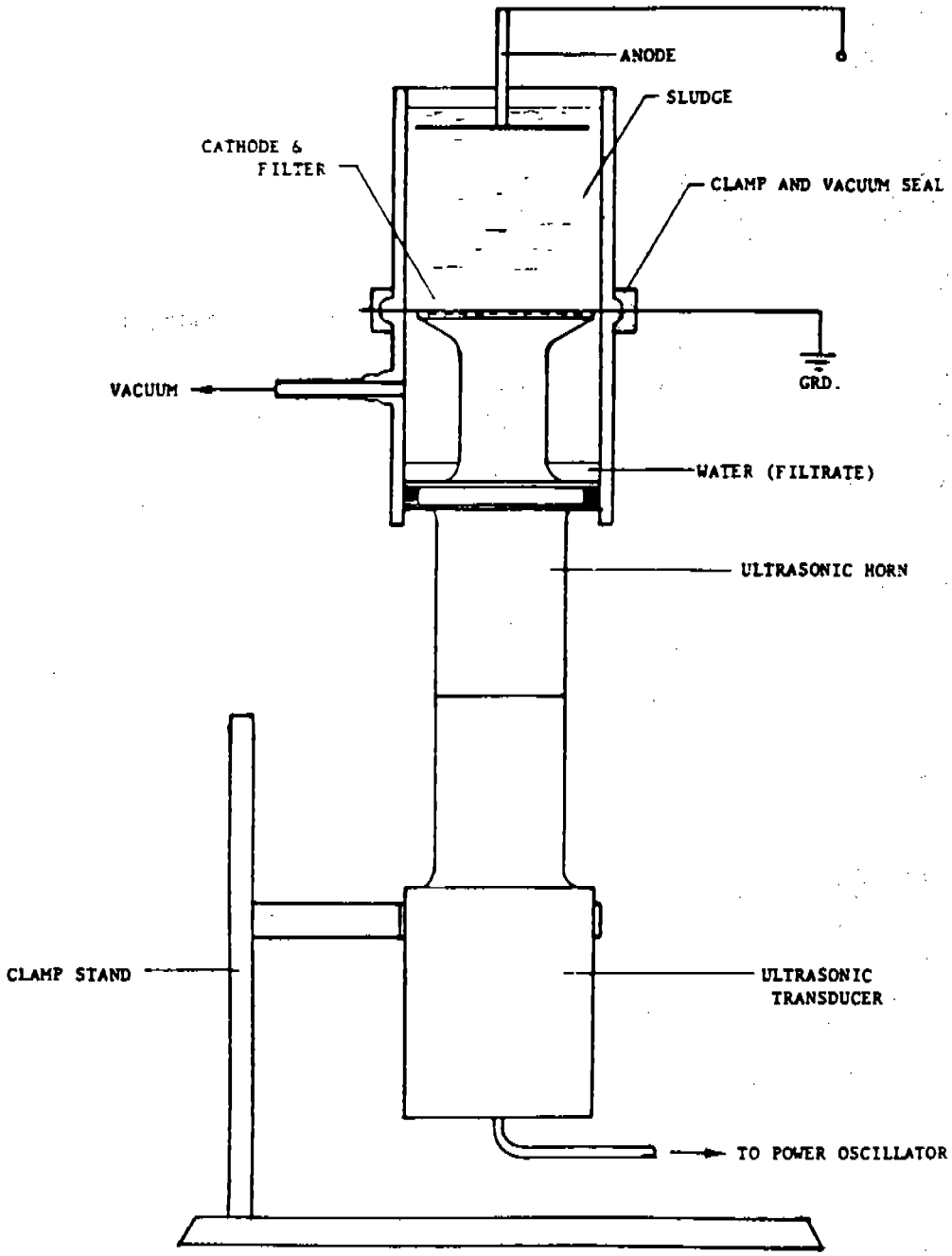


FIGURE 1. SCHEMATIC OF MODIFIED BATCH LABORATORY ELECTROACOUSTIC DEWATERING SYSTEM

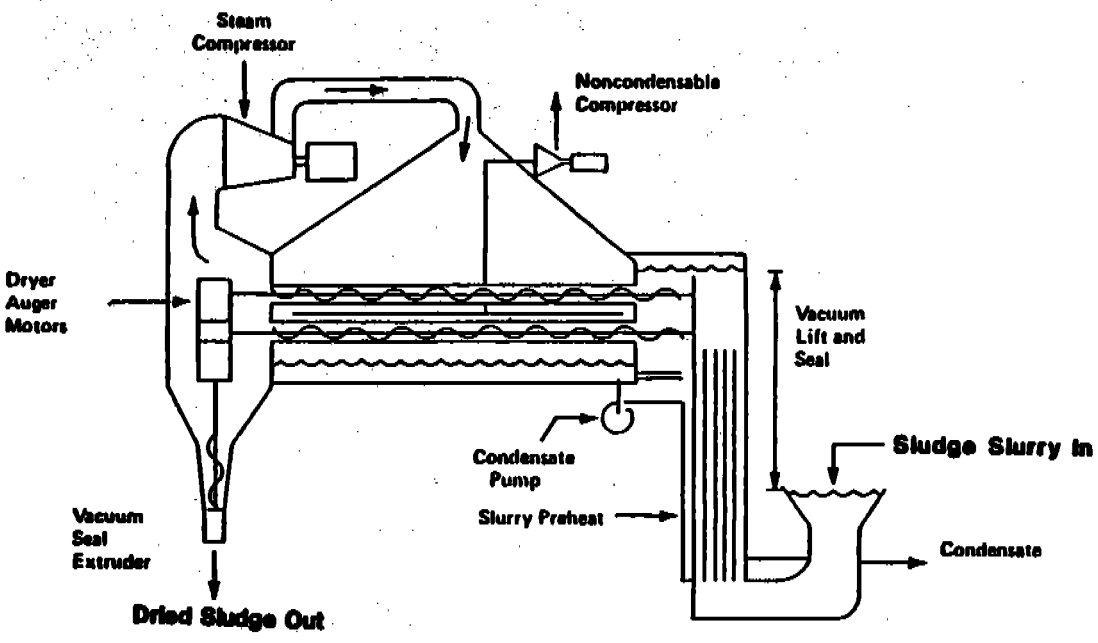


Figure 2.Low-Temperature Contact Dryer/Heat Pump Concept

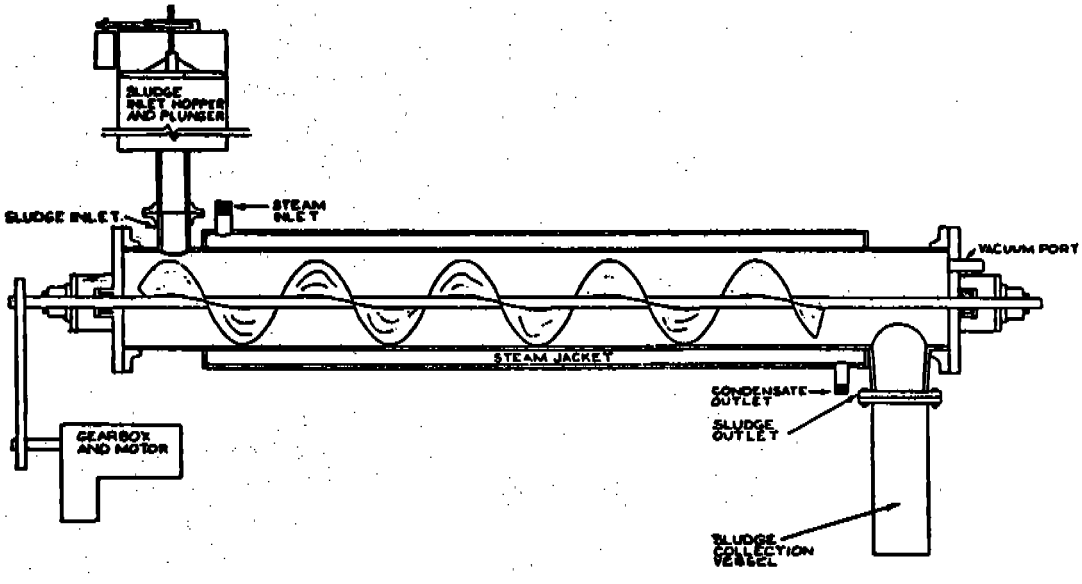


Figure 3.Contact Dryer Test Apparatus

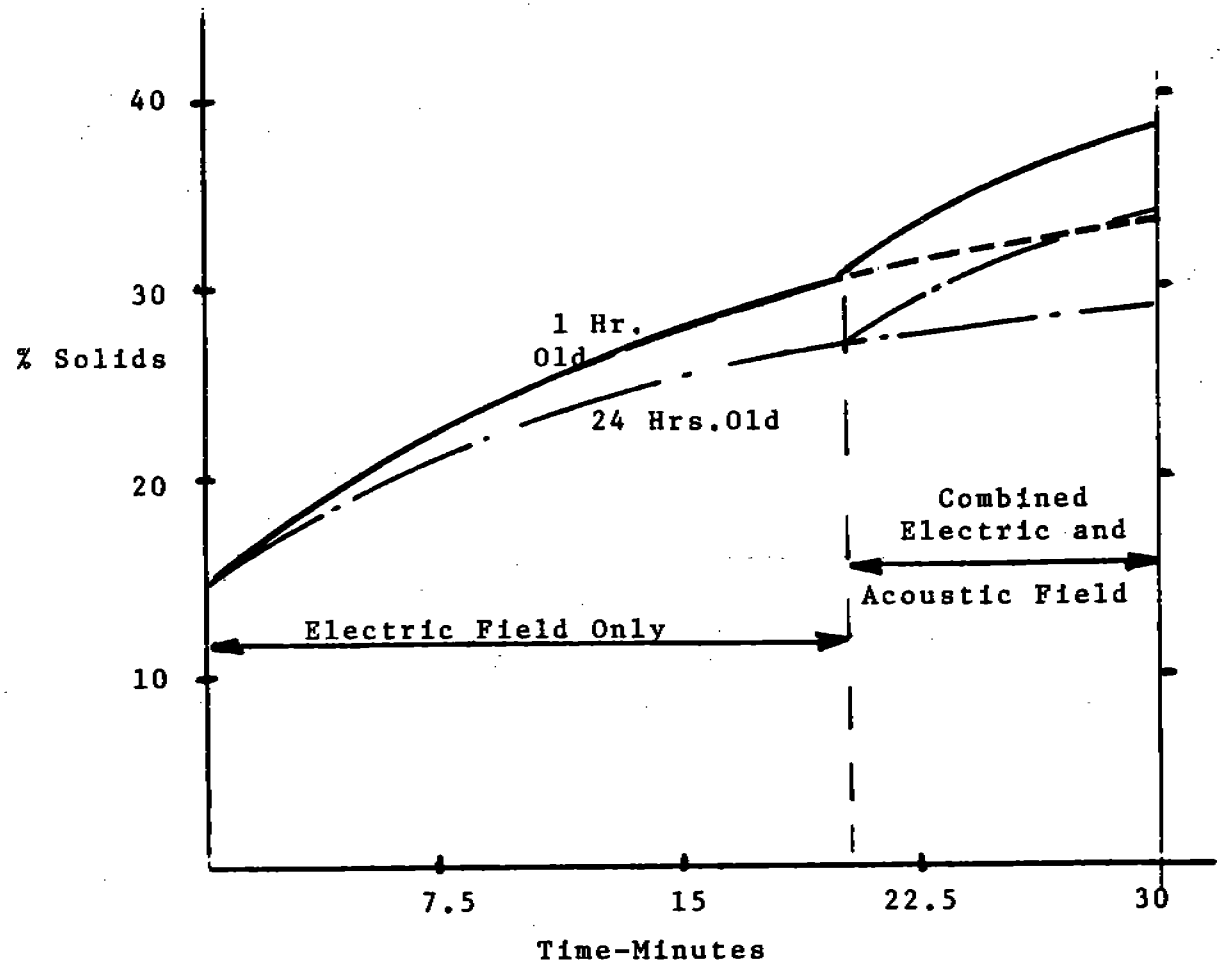


Figure 4. EAD Process Performance

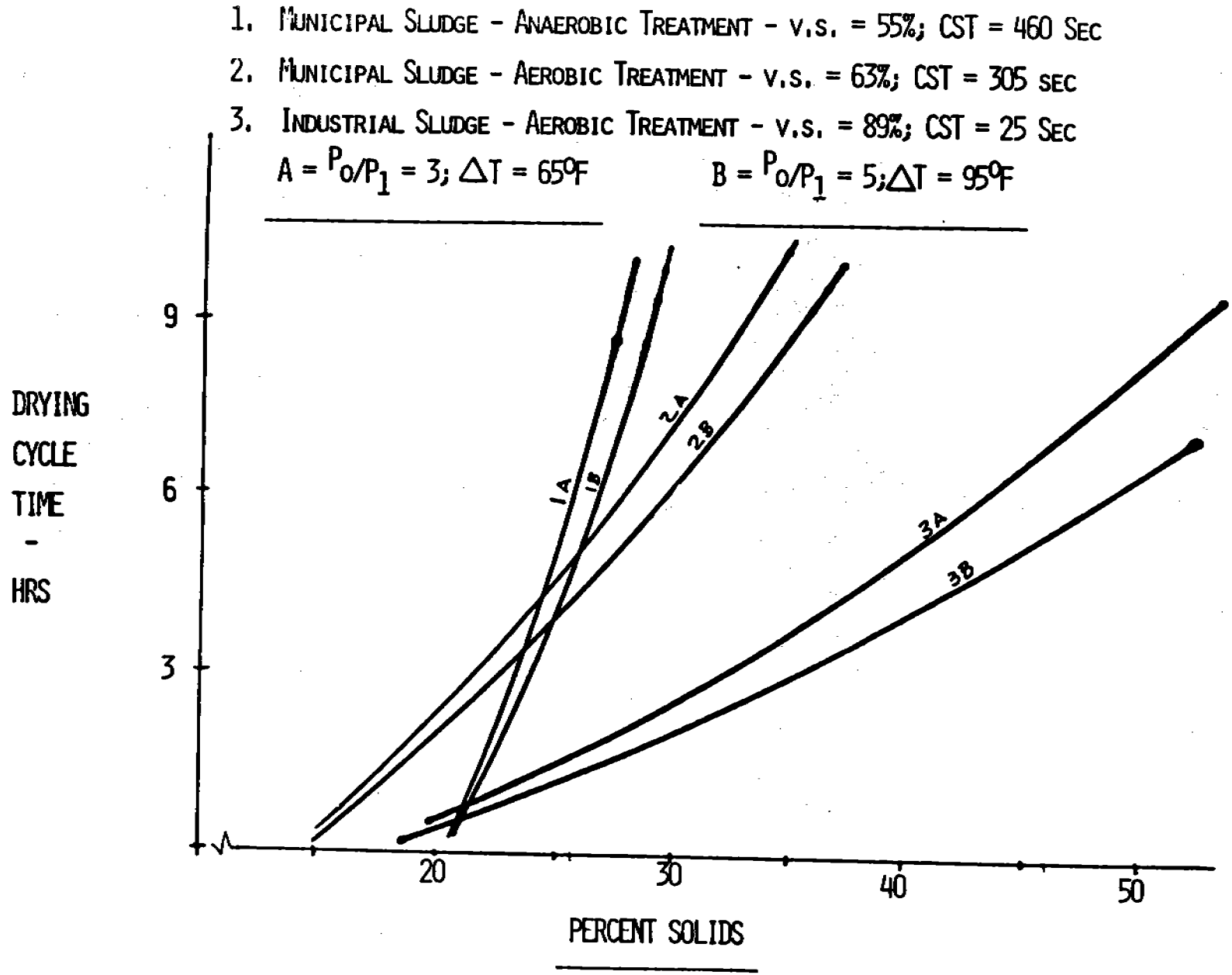


Figure5- Low-Temp., Low Press. Contact Dryer Screen Test Results with Three Types of Sludge

RESEARCH INTO FACTORS DETERMINING ANAEROBIC DIGESTER PERFORMANCE

R.E.SPEECE
ENVIRONMENTAL STUDIES INSTITUTE
DREXEL UNIVERSITY
PHILADELPHIA, PA. 19104

ABSTRACT

This research falls into 3 phases. The first phase was a study of 30 municipal sludge digesters and development of diasgnostic techniques to assay the activity and efficiency of their performance. The second phase was the operation of pilot digesters at a municiapal plant to assay the effectiveness of trace metal supplementation on digestion performance at reduced detention times. The third phase was the determination of the minimum concentration of eight essential inorganic nutrients for high rate acetate conversion to methane (e. g. 30-40 g/l-d) at low SRT (e. g. 5 days). In the first phase, 8 of the 30 sludges showed over 20% stimulation in gas production rate when iron, cobalt and/or nickel was supplementaed and acetate was unlimiting. Nine of the 30 sludges showed over 20% stimulation with trace metal supplementation when propionate was unlimiting. In the secomd phase, using domestic sludge in digesters mixed once per day, there was a statistically significant benefit of supplementing trace metals at 10, 6.7 and 5 days SRT. In the third phase, the minimum concentration of the following nutrients was determined which could sustain 30 - 40 g/l-d conversion of acetate to methane at 5 days SRT (mg/l): N-100, P-4, S-10, Ca-5, Mg-1, Fe-1, Co-0.1 and Ni-0.2. The predominance shift from the slow-growing Methanothrix to the faster-growing Methanosarcina could only be achieved when trace metals were supplemented.

RESEARCH INTO FACTORS DETERMINING ANAEROBIC DIGESTER PERFORMANCE

PHASE 1 - DIAGNOSTIC ASSAY TECHNIQUES

Introduction

Anaerobic sludge digesters in conventional municipal wastewater treatment plants process about two-thirds of the influent flow BOD. Commonly one-third of the influent BOD is removed by primary sedimentation and pumped to the digester. Approximately half of the remaining BOD is synthesized into excess sludge in secondary treatment and this is also pumped to the digester. In spite of the key role played by anaerobic sludge digestion, this process commonly receives less operational attention than the activated sludge process. Gas production, solids content in and out, pH, volatile acids concentration and alkalinity are the commonly measured parameters.

With the activated sludge process, common activity assays are conducted. For example, the residual BOD in the effluent is determined. Some plants also measure the specific oxygen uptake rate (SOUR) of the mixed liquor in the aeration tank. This paper is intended to demonstrate that anaerobic sludge digesters also lend themselves to a number of diagnostic activity assays whereby the process can be monitored.

Since sludge digesters contain such a high fraction of inert solids, analysis of solids per se is not a meaningful measure of biomass. However, the acetate and propionate utilization rates are crucial factors in the digestion process and these parameters can be assayed even though the responsible biomass cannot be easily measured. The bioavailability of iron, cobalt and nickel has been shown to limit the rate of volatile acids conversion in some digesters. Assays of sludge samples spiked with these metals can also be made to assay trace metal limitations in the process. Two more diagnostic assays which are quite simple to run are to 5 and 30 day Biochemical Methane Potentials (BMP_5 and BMP_{30}).

In this study, the following diagnostic activity assays were made on sludge samples from anaerobic sludge digesters at 30 cities in the United States:

Maximum Potential Acetate Utilization Rate (MPAUR)
Maximum Potential Propionate Utilization Rate (MPPUR)
Bioavailability of Iron, Cobalt and Nickel
BMP_5
BMP_{30}

RESULTS AND DISCUSSION

BMP_5

Low BMP_5 values reflect stable sludges while high BMP_5 values indicate more putrescible sludges which have the potential for producing more methane. One volume of methane per volume of sludge at 35°C is equivalent to a COD reduction of 2400 mg/l (0.15 lb COD/ft^3). The range of BMP_5 values was 0.1 to 5.4 V CH_4/V of sludge with an average of 0.9 ± 0.9. By eliminating these two extreme values, the new range was 0.2 to 1.6 and the new average was 0.7 ± 0.5 V CH_4/V of sludge. This compares with an average gas yield from raw sludge of 15.8 ± 7.5 V CH_4/V of sludge for each of the cities in this study which metered gas production. This corresponds to a BMP_5 removal of 96%.

BMP_{30}

The range of BMP_{30} values was from 0.1 to 8.7 with an average of 2.1 ± 1.8 V CH_4/V of sludge. The exclusion of these extreme values gave a new range of 0.5 to 6.7 and a new average of 1.9 ± 1.3 V CH_4/V. This would indicate that with the average sludge assayed 1.9 volumes of potential methane remained in the sludge when it was disposed of, or 4700 mg/l of biodegradable COD remained which potentially could be converted to methane.

BMP_{30}/(Volume of CH_4/VolumeRaw Sludge)

When the BMP_{30} is divided by the actual volume of CH_4 produced/volume of raw sludge, it gives a rough quantitative measure of the ratio of gas production potential remaining in the sludge compared to the gas actually produced by the sludge. The range of this ratio was 0.01 to 0.82 with an average of 0.16 ± 0.19. Excluding these two extreme values gave a new range of 0.03 to 0.50 with a new average of 0.13 ± 0.13, indicating that about 87% of the BMP_{30} was converted to methane in the average digester.

Maximum Potential Acetate Utilization Rate

The range of MPAUR noted was 0.1 to 2.5 V CH_4/V of sludge-day with an average of 0.95 ± 0.53. Excluding these two extremes, the new range was 0.2 to 2.0 and the new average was 0.93 ± 0.44 V CH_4/V of sludge-day.

Maximum Potential Propionate Utilization Rate

Conversion of propionate to acetate and hydrogen is a crucial step in anaerobic digestion. Propionate at elevated concentrations has been noted in malfunctioning digesters and thus its rate of utilization is useful as a diagnostic technique to evaluate the condition of a digester. The range of MPPUR was 0 to 0.4 with an average of 0.18 ± 0.14 V CH_4/V of sludge-day.

Safety Factor

The "Safety Factor" (MPAUR/actual gas production rate) was calculated in the following manner: the MPAUR was divided by the actual methane production rate of the digesters (calculated from treatment plant records). The results were that the values ranged from 1.0 to 8.5. Ignoring the 8.5 value gave an average of 1.8 ± .64. It should be pointed out that the digesters having a safety factor of 8.5 were at a temperature of 16-27°C, causing the sludge to have a very low activity compared to the MPAUR assay run at 35°C.

Trace Metal Stimulation of the MPAUR

Table 1 summarizes the sludge samples which gave 20% or more increase in gas production in the MPAUR assays when trace metals were supplemented. It is significant that trace metal stimulation of the MPAUR was noted for 8 out of the 30 digesters sampled, indicating that for these sludges the conversion of acetate to methane was rate-limited by bioavailability of trace metals under unlimiting acetate concentrations. (It should be noted that hydrolysis and/or acid production could still be the rate controlling step if the digester was lightly loaded.)

TABLE 1
MPAUR STIMULATION BY TRACE METAL SUPPLEMENTATION (% Stimulation)

CITY	Fe	Co	Ni	Fe/Co/Ni
Albuquerque, NM	23	-	23	50
Baltimore, MD	167	133	111	111
Calumet,IL	-	-	29	54
Cheyenne-Crow Crk,WY	-	-	-	27
Cheyenne- Dry Crk, WY	-	-	-	27
Cincinnati, OH	32	-	-	-
Littleton, CO	22	-	-	-
San Antonio,TX	-	-	-	20

The soluble concentration of iron, cobalt and nickel are all relatively high, perhaps causing a tacit assumption that soluble trace metals are bioavailable. However, such an assumption may not be well-founded for a strong chelator such as EDTA can keep iron in the soluble form, but it is not bioavailable to the methanogens according to our laboratory studies. Thus, it may be hypothesized that there are naturally formed chelators of trace metals in the sludge that bind the metals so tightly that they are not bioavailable. This hypothesis is based upon the observation that even though soluble iron, cobalt and nickel concentrations appear to be ample in the sludges, yet their supplementation caused stimulation in the eight cases noted.

Trace Metal Stimulation of the MPPUR

Table 2 summarizes the sludge samples which gave 20% or more increase in gas production in the MPPUR assays when trace metals were supplemented. Nine of the 30 samples gave MPPUR which were stimulated by trace metal supplementation.

TABLE 2
MPPUR STIMULATION BY TRACE METAL SUPPLEMENTATION (% Stimulation)

CITY	Fe	Co	Ni	Fe/Co//Ni
Albuquerque, NM	33	-	58	142
Calumet,IL	75	75	125	200
Cheyenne-Crow Crk, Wy	27	27	31	62
Cheyenne-Dry Crk, Wy	-	38	54	108
Chicago, IL	22	-	37	-
Egan, IL	35	35	39	78
El Paso, TX	26	22	33	67
Pueblo, CO	53	53	53	124
San Antonio,TX	24	-	31	26

Hydrogen Sulfide

The H_2S content of digester gas as measured by the author during the plant visit, varied from

non-detectable to 6200 ppm. The average H_2S content was 2200 ± 1800 ppm, as shown in Table 6. The solubility of H_2S is approximately 2000 mg/l un-ionized H_2S in the liquid per atmosphere of H_2S partial pressure in the head gas. Thus 2200 ppm H_2S in the head gas would be about 4 mg/l of un-ionized H_2S in the liquid phase. Since the pK of H_2S is 6.85, which is below the actual pH of most digesters, the ionized H_2S would be somewhat greater than 4 mg/l for a total average soluble sulfide in excess of 8 mg/l in the liquid phase.

There is no apparent correlation between digester operational characteristics and H_2S content. For instance, Chicago Southwest conditioned its waste activated sludge with ferric chloride prior to vacuum filtration. The filter cake, laden with iron salts, was then diluted to 5.5% and fed to the digesters. There was no detectable H_2S in their digester gas, yet the corresponding methanogenic activity was among the highest recorded for all the cities. In contrast, the Terminal Island plant in Los Angeles had 6200 ppm H_2S in their digester gas and their records indicated that as high as 10,000 ppm H_2S had occured, with no indication of inhibition of methanogenic activity.

Design Digester Capacity

Digester capacity provided at the cities sampled varied from .01 to .40 MG per MGD of plant raw wastewater flow. The average was 0.14 ± .09 MG per MGD of plant flow which would be equivalent to 54 liters (1.9 ft^3) of digester capacity per capita if the per capita flow was 375 liters/day (100 gpd).

Operational Characteristics

The volume of raw sludge pumped to the digesters per MGD of raw wastewater plant flow varied from 0.0011 to 0.011 V/V (1,100 to 11,000 gal/MGD). The average was 0.0043 ± 0.0028 V/V (4300 ±2800 gal raw sludge/MGD). These data need to be tempered by the fact that some plants treat sludges from their own operations plus satellite plants which had no sludge handling facilities.

Digester gas production varied from .0165 to 0.175 V gas/V of raw wastewater flow (2200 to 23,300 ft^3 per MG). The average was .066 ± .046 V gas/V raw wastewater(8800 ± 6100 ft^3 gas/per MG).

The unit gas production rate per volume of digester per day varied from 0.13 to 2.1. The average was 0.64 ± 0.50 volumes of digester gas per volume of digester per day. If the gas composition was 67% methane, this would represent a COD destruction rate of about 1 g/l-day (0.06 lbs COD destroyed per ft^3 - day).

The volume of digester gas produced per volume of raw sludge fed is due to the product of raw sludge concentration and degree of digestion. One city reported as low as 2.1 volumes of digester gas per volume of raw sludge feed, where it appears there may have been a major problem of gas being lost through leakage from the digesters at this installation because the raw feed sludge was of typical solids concentration and the BMP_5 and BMP_{30} were also typical, indicating proper digestion of the sludge had occured. Omitting this value, the range was 6.5 to 35.6 volumes of digester gas per volume of raw sludge fed while the average was 15.8 ±7.5. Gas meters were a common maintenance problem.

PHASE TWO - TRACE METAL STIMULATION OF SLUDGE DIGESTION

EXPERIMENTAL DESIGN

Domestic sludges were digested at HRT/SRT of 5,6,7, 10 and 20 days with and without the supplementations of iron, cobalt and nickel. Single and two phase digestion were also compared.

RESULTS AND DISCUSSION

Data Summary of 5 day SRT

	None 5.0	All 5.0	None2.5(2nd stg.)	All 2.5(2nd stg.)
GAS (l/D,PH>6.4	56–16	70–11	82–16	134–135
% CH4	38	40	46	48
Temp (°C)	88–2	90–2	95–3	91–3
pH	6.4–0.5	6.4–0.4	6.2–0.6	6.2–0.4
Total Sol. (g/l	37.4	37.0	---	---
%Volatile	68	66	---	---
Fe (mg/l	67	68	---	---
Ac (mg/l)	4.000	4.000	---	---
Pr. (mg/l)	1.500	1.000	---	---

Data Summary of 6.7 Day SRT

	None 6.7	All 6.7	None 4.2	All 4.2
Gas (l/D.pH>6.4)	78–20	91–15	61–25	85–34
% CH4	58	66	50	50
Temp (°C)	89–2	91–2	91–2	93–4
pH	6.8–0.3	6.8–0.2	6.6–0.5	6.6–0.5
Total Sol.(g/l)	36.0	31.5	30.9	33.6
% Volatile	65	64	65	67
Fe(mg/l)	3.2	1.9	3.1	5.1
Ac.	400	90	450	3,200
Pr.	1.700	1.000	1.700	1.500

Data Summary of 10 Day SRT

	None 10	All 10	None 7.5	All 7.5
Gas (l/D)	144–50	163–50	177–33	194–35
Alk. (mg/l)	3134–218	3242–153	3258–220	3283–149
Temp(°C)	93–15	93–3	95–1.3	94–4
pH	7.2	7.2	7.3	7.2
Total Sol. (g/l)	27.1–1.6	26.4–0.5	26.3–2.7	27.1–1.1
% Volatile	60%–3	61%–3	59%–3	61%–2
Acetic (mg/l)	34–39	22–13	153–203	182–201
Propionic (mg/l)	0	0	59–107	75–100
Fe (mg/l)	0.8–.2	0.8–.2	0.9–0.4	0.8–0.3

SUMMARY

The two phase systems operated less stably than the single phase systems at both the 5 and 6.7 day SRT. Analysis of the gas production data shows that trace metals have a statistically significant effect on gas production in

the two phase systems at both 5 and 6.7 day SRT.

These results indicate that at 10, 6.7 and 5 days SRT, there is a statistically significant benefit of trace material supplementation in the anaerobic digestion of sludges from a municipal waste water treatment plant. This is considered to be a very important finding of considerable consequence to anaerobic practice.

The concentration of volatile acids were low in both the single and two phase systems. This indicates that acid conversion to methane is not the critical rate-limiting step at low loading rate/detention times of 10 and 20 days. Analysis of the gas production data shows that trace metals
have a statistically significant effect on gas production in the two phase systems at both 10 and 20 day SRT. However, there is no clear pattern to indicate superior performance of the single or two phase systems.

Under continuous mixing conditions, the stimulation by trace metal supplementation could not be demonstrated statistically. This needs further study.

PHASE 3 MINIMUM INORGANIC NUTRIENT REQUIREMENTS FOR HIGH RATE LOW SRT METHANE FERMENTATION

OBJECTIVE

In this study, we define the minimum concentration of eight inorganic nutrients: - nitrogen, phosphorus, sulfur, calcium, magnesium, iron, cobalt, nickel - required to support a high acetate utilization rate (AUR) of 30 to 40 g/l-d with what is considered for methanogens to be a rather low solids retention time (SRT) of five days.

All of the studies performed for this research utilized acetate enrichment cultures. An acetate enrichment culture dominated by *Methanosarcina* species was principally used as an inoculum source in order to quantitatively define nutrient requirements for high rate conversion of acetate to methane. In the study of a nutrient, it is desirable to keep nutrients other than the limiting one in excess. For this reason, the cultivation system used for this study was a completely stirred tank reactor (CSTR) "pH stat" that could maintain reasonably constant pH and unlimited levels of the carbon-energy source, acetate, in conjunction with proper supplementation of alkalinity. A pH controlled pump automatically maintained the acetate concentration between 2000 and 3000 mg/l. This system overcomes the disadvantage of the general batch method in which high acetate levels can be maintained only for a short period, and of the general chemostat system in which high loading rates and high substrate concentrations are difficult to achieve when the substrate is an acid.

The pH stats were operated continuously . Since most anaerobic digesters in the field are operated in a continuous mode, it was felt that continuous flow experiments would provide more realistic information than batch experiments. All experiments were conducted under reasonably constant environmental conditions: pH of 6.8 and temperature of 35 ^{o}C. For these experiments, SRT/HRT was fixed at 5 days, except the last study of 2.5, 3.3, 5.0, 10 and 20 days . Acetate utilization rates (AUR) were monitored every day, and biomass and nutrient concentrations were measured periodically.

To test the quantitative requirements for inorganic nutrients a classical, one factor design was employed in which concentrations of the one nutrient assayed were varied, and the concentration of others was kept constant. The eight nutrients examined were presumed to be the most important ones: nitrogen (as ammonia-nitrogen), phosphorus (as phosphate), sulfur (as sulfide), calcium, magnesium, iron, nickel and cobalt. For this study, the nutrient assayed was deleted from the basal culture media shown in Table 3, and added separately and directly to the pH stats.

From the results obtained in the preceding experiments, the lowest concentrations of the eight inorganic nutrients were chosen which would support near maximum acetate utilization rates of approximately 30 to 40 g/l-d or more AUR at 5 days SRT/HRT. The combination of the concentrations of these nutrients, called minimum nutrient requirements, which could support the near maximum AUR was confirmed in this experiment. The concentrations of nutrients other than the eight nutrients were kept the same as those in the basal culture media.

In a final experiment, SRT/HRT was varied (2.5, 3.3, 5, 10 and 20 days), and the effects of these retention times were examined. Again, the eight nutrients were deleted from the culture media, and were added separately directly to the CSTR reactors.

Table 3 Composition of Drexel Media

Constituent	Concentration (mg/l)
NH_4Cl	1,200
$MgSO_4 \cdot 7H_2O$	400
KCl	400
$Na_2S \cdot 9H_2O$	300
$CaCl_2 \cdot 2H_2O$	750
$(NH_4)_2HPO_4$	80
$FeCl_2 \cdot 4H_2O$	40
$CoCl_2 \cdot 6H_2O$	10
KI	10
$(NaPO_3)_6$	10
$MnCl_2 \cdot 4H_2O$	0.5
NH_4VO_3	0.5
$CuCl_2 \cdot 2H_2O$	0.5
$ZnCl_2$	0.5
$AlCl_3 \cdot 6H_2O$	0.5
$NaMoO_4 \cdot 2H_2O$	0.5
H_3BO_3	0.5
$NiCl_2 \cdot 6H_2O$	0.5
$NaWO_4 \cdot 2H_2O$	0.5
Na_2SeO_3	0.5
Cysteine 10	
$NaHCO_3$ 6,000	

Table 4 Approximate minimum nutrient requirements to support near 30 g/l-d or more AUR of acetate enrichment at 5 days SRT/HRT

Nutrient	Concentration added (mg/l-d)	Concentration in reactor (soluble) (mg/l)
NH_4-N	100	70
PO_4-P	4	0.1
S	10	4
Ca	5	30
Mg	1	3
Fe	1	0.5
Ni	0.2	<0.01
Co	0.1 - 0.2	0.05

RESULTS AND DISCUSSION

The average AUR and nutrient concentration in the reactor are plotted as a function of nutrient concentration

added in Figs. 1 to 8. The concentration of nutrients is expressed as mg/l-d, e.g. mg of nutrient per liter of reactor volume per day.

The AUR increased relatively linearly as the nutrient concentration added increased, and gradually saturated resulting in 30-40 g/l-d AUR at sufficiently high levels of nutrients. To support 30 g/l-d or more AUR of acetate enrichment at 5 days SRT/HRT, the minimum amount of eight nutrients to be added and the actual concentration in the reactor are approximately as shown in Table 4.

Considering the general importance and the physiological functions reported for methanogens, all eight nutrients are presumed to be essential. From the relatively linear response to the nutrient concentration added and nearly washout condition at 0 mg/l-d addition (about 3 g/l-d AUR or less except for Ca and Co), it can be said that the essentiality of those nutrients for the acetate enrichment was demonstrated in this experiment. The contamination of inorganics in the culture media accounts for the presence of active biomass even without the direct supply of a given nutrient, but the methanogens might also be able to adapt to extremely low concentrations of the nutrients.

Cost of Nutrient Supplementation

Table 5 shows the cost of nutrient supplemetation .

Table 5 Estimated cost of nutrient supplementation at 5 days SRT/HRT (the cost of nutrients is from Chemical Reporter, June 1987)

Element	Requirement (mg/g Ac)	Cost of nutrient ($/lb.)	Cost of nutrient per ton of acetate ($/ton acetate)
NH_4-N	3.3	0.30	2.20
PO_4-P	0.10	0.40	0.09
S	0.33	0.60	0.44
Ca	0.13	0.31	0.09
Mg	0.018	0.50	0.02
Fe	0.023	0.75	0.04
Ni	0.0040	8.00	0.01
Co	0.0030	20.00	0.13
		Total $	3.08

ACKNOWLEDGEMENT

This research was mainly funded by a contract with Argonne National Laboratory, #31-109-38-7169, with Mr. C. V. Pearson , Contract Officer.

FUTURE WORK

- MINIMUM TRACE METAL REQUIREMENTS FOR COMPLEX FEEDS.
- ROLE OF TRACE METALS IN AVERTING DIGESTER FAILURE.
- DETERMINATION OF CAUSE OF CHRONICALLY HIGH VOLATILE ACIDS.

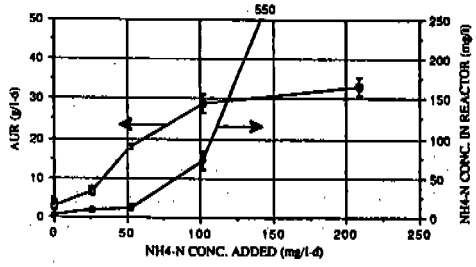


Fig. 1 AUR and NH4-N conc. in reactor at different NH4-N conc. added
Average and s.d. (shown with the error bar) during the data taking period

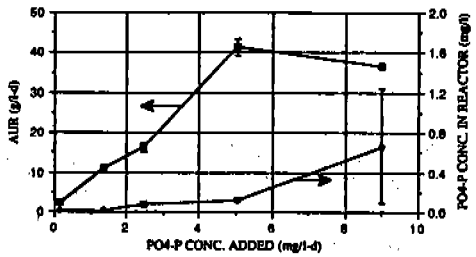


Fig. 2 AUR and PO4-P conc. in reactor at different PO4-P conc. added
Average and s.d. (shown with the error bar) during the data taking period

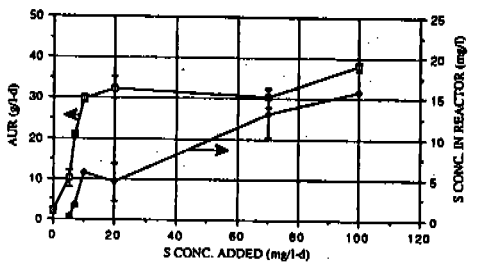


Fig. 3 AUR and S conc. in reactor at different S conc. added
Average and s.d. (shown with the error bar) during the data taking period

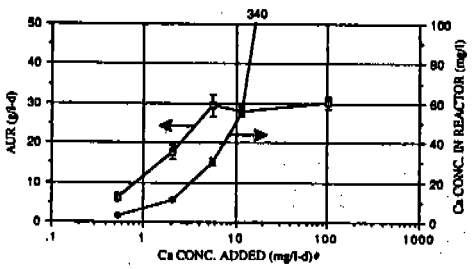


Fig. 4 AUR and Ca conc. in reactor at different Ca conc. added
Average and s.d. (shown with the error bar) during the data taking period

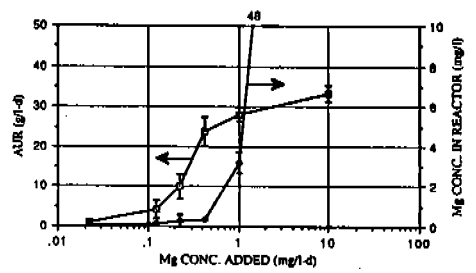


Fig. 5 AUR and Mg conc. in reactor at different Mg conc. added
Average and s.d. (shown with the error bar) during the data taking period

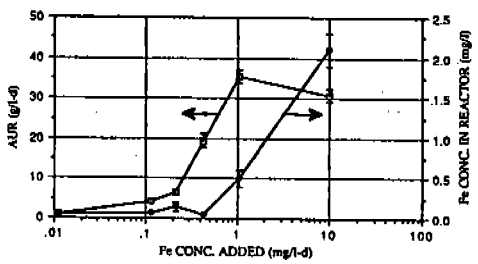


Fig. 6 AUR and Fe conc. in reactor at different Fe conc. added
Average and s.d. (shown with the error bar) during the data taking period

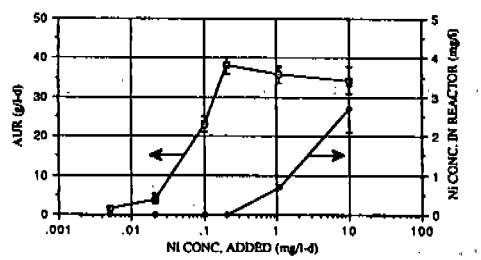


Fig. 7 AUR and Ni conc. in reactor at different Ni conc. added
Average and s.d. (shown with the error bar) during the data taking period

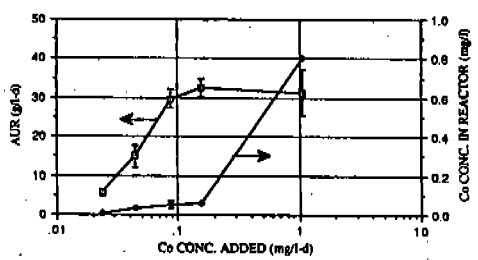


Fig. 8 AUR and Co conc. in reactor at different Co conc. added
Average and s.d. (shown with the error bar) during the data taking period